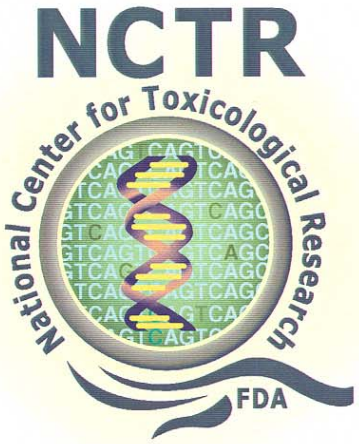
National Center for Toxicological Research
Research Accomplishments and Plans

FY 2002-2003



Jefferson Laboratories of the FDA

Leaders in Health Science Research for the FDA

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FDA

Preface

The National Center for Toxicological Research (NCTR) plays a critical role in the U.S. Food and Drug Administration (FDA) and Department of Health and Human Services (DHHS) mission to protect public health. The Center, a component of the Jefferson Laboratories of the FDA, is located in Jefferson, Arkansas, approximately 30 miles south of Little Rock.

The mission of NCTR is to conduct peer-reviewed scientific research that supports the FDA's current mission and anticipates future regulatory needs. This involves research specifically designed to define biological mechanisms of action underlying the toxicity of products regulated by the FDA and the development of improved methods for assessment of human exposure, susceptibility, and risk. In addition to its FDA support, NCTR leverages its resources by conducting integrated research programs with other FDA centers, the Office of Regulatory Affairs (ORA), and through collaborative agreements with other government agencies, academia, and industry. NCTR receives guidance and advice on the relevance and quality of its research programs from an extramural Science Advisory Board, liaison members from each of the other FDA centers, the ORA, and other stakeholders.

The NCTR views its public health role as a key element in the development and modification of toxicology safety standards through the application of innovative scientific research. New health concerns, such as bovine spongiform encephalopathy (BSE), AIDS, pediatric initiatives, skin cancer, antibiotic resistance, counter terrorism and emerging foodborne pathogens, in addition to traditional concerns, are challenging the conventional ways in which the regulatory agencies (both national and international) set safety standards designed to protect public health. Additionally, the NCTR is a participant in national and international consortia that are developing standards for using emerging genomic technologies and standards for interpreting the data derived from these technologies. Examples of how NCTR is supporting and meeting standard-setting challenges of the FDA include:

- Publishing an on-line scientific journal entitled *Regulatory Research Perspectives*, which highlights some of the latest research topics in the scientific regulatory arena.
- Refining rapid detection methods for potential bioterrorism agents.
- Developing microarray/proteomic technology to provide high-volume screening of biomarkers for susceptible subpopulations and evaluate the effects of chemical toxicants on gene expression and protein profiles.
- Introducing the knowledge of new genetic systems, specifically transgenic systems and data, into the application review process.
- Developing computer-based models to predict the impact of increased exposure to toxic compounds on public health.
- Conducting studies on FDA-regulated compounds to relate the mechanism(s) by which a chemical causes toxicity to the biological outcome.
- Developing and/or modifying standards to better suit the regulatory needs of the DHHS/FDA for food safety.

- Developing methods and building biological dose-response models to quickly and accurately predict risks associated with antimicrobial resistance and foodborne pathogens/contaminated foods, dietary supplements, and genetically modified foods.

Other important areas of research supported in part by external funding include identification of the effects of anticonvulsants on complex brain functions in non-human primates, antibiotic resistance associated with competitive exclusion products, and development of risk assessment tools to better extrapolate animal toxicity data to humans.

Perhaps of greater importance to our research accomplishments was the benefit gained by sharing knowledge through collaborations with scientific staff of other government, academic, and industrial institutions. I am proud to present this report that summarizes these and other NCTR research accomplishments and plans for the fiscal years 2002-2003.

Daniel A. Casciano, Ph.D.
Director, NCTR

Science Advisory Board

Function

The NCTR Science Advisory Board (SAB) advises the Director in establishing, implementing and evaluating the research programs that assist the Commissioner of the Food and Drug Administration (FDA) in fulfilling regulatory responsibilities. This external body of recognized scientific experts is a key component of the review and planning process, and helps to ensure that the research programs at NCTR are scientifically sound and pertinent to the FDA.

FY 2002 Accomplishments

A full meeting of the Board was held August 8-9, 2002.

The Board received a report from the Site Visit Team's (SVT) review of the Division of Chemistry. The SAB accepted the report and addressed issues raised by the SVT and its recommendations. The division director provided an initial response to the SVT report. The Director and staff of the Chemistry Division will provide an in-depth response at the next full meeting of the SAB.

The Board revisited the proposal that it consider establishing a subcommittee on scientific opportunities to improve regulatory science through collaborations with external stakeholders. A report was provided to the Board on the activities of an existing subcommittee with a similar focus (FDA Center for Drug Evaluation and Research [CDER], Advisory Committee for Pharmaceutical Science [ACPS], Nonclinical Studies Subcommittee [NCSS]). A proposal was made to the Board that it consider the migration of the NCSS from the CDER, ACPS to the NCTR, SAB. The Board requested additional information, and elected to postpone making a decision pending consideration of the additional information they sought.

The Board was introduced to the directors of the newly established NCTR Centers of Excellence: the Functional Genomics Center, Phototoxicology Center, Structural Genomics Center, and Toxicoinformatics Center. Each of the directors provided the Board with a synopsis of what their center's function would be relative to research at the NCTR and other FDA research components.

The site visits reports and the minutes of the SAB meetings can be accessed at http://www.fda.gov/nctr/science/committees/committees.htm.

Science Advisory Board Membership Roster

NAME/TITLE	AFFILIATION	TERM ENDS	EXPERTISE
Dr. Daniel Acosta, Jr.* Dean, College of Pharmacy	University of Cincinnati	6/30/03	Pharmacology and Toxicology
Dr. Nancy Ann Gillette Sr. Vice President Sierra Biomedical	Charles River Laboratories	6/30/03	Veterinary Medicine and Pathology
Dr. Jerry Kaplan Associate Dean for Research	University of Utah School of Medicine	6/30/04	Molecular Biology
Dr. John Groopman	Bloomberg School of Public Health Department of Environmental Health Sciences	6/30/06	Toxicology
Dr. Pat R. Levitt	Vanderbilt University, John F. Kennedy Center for Research and Human Development	6/30/06	Neurobiology
Dr. E. Albert Reece Vice Chancellor and Dean	University of Arkansas College of Medicine	6/30/06	Physician
Dr. Alberto Luis Rivera-Rentas	School of Environmental Affairs, Ana G. Mendez University System	6/30/06	Neurobiology Electrophysiology

*Chair

FDA Coordination Activities—Safety Testing

Function

The NCTR Office of Washington Operations serves as the Agency coordinator for activities of the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) and represents the Agency on Economic Cooperation and Development (OECD) matters related to the Test Guidelines Program of OECD.

The ICCVAM coordinates and advises on interagency issues on development, validation, and regulatory acceptance of improved methods for acute and chronic safety testing, including alternative methods, and the national and international harmonization of such methods. Congress recently enacted the ICCVAM Authorization Act (December 19, 2000) "to establish, wherever feasible, guidelines, recommendations, and regulations that promote the regulatory acceptance of new or revised scientifically valid toxicological tests that protect human and animal health and the environment while reducing, refining, or replacing animal tests and ensuring human safety and product effectiveness." As a result, ICCVAM, which was initially assembled as an *ad hoc* committee and had evolved to a standing committee, became a permanent committee.

ICCVAM's charge includes:

- Promote the scientific validation and regulatory acceptance of new/improved alternative test methods.
- Coordinate the review/evaluation of new/revised alternative test methods of interagency interest.
- Facilitate and provide guidance on test method development, the validation process, validation criteria, regulatory acceptance criteria, and submission requirements.
- Provide recommendations to Federal agencies on the validation status of test methods and their regulatory suitability.
- Facilitate interagency regulatory acceptance and promote international harmonization and adoption of scientifically validated test methods.
- Facilitate awareness of and training for accepted test methods (end-users, regulators).

ICCVAM is currently revising its operating procedures to expand its activities and improve the efficiency of the validation and acceptance of new test methods for regulatory purposes.

The Scientific Advisory Committee for the Validation of Alternative Methods (SACATM), which advises ICCVAM and its operational center, the National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), has been established and chartered (December 18, 2001). The SACATM provides advice regarding such issues as:

- Priorities and opportunities for alternative test methods that may provide improved prediction of adverse health effects compared to currently used methods or advantages in terms of reduced expense and time, reduced animal use, and reduced animal pain and distress.

- Development and implementation of more effective and efficient processes for determining the scientific validity and acceptability of proposed new test methods.
- Fostering more effective and productive interactions between Federal agencies and other involved stakeholders, including test method developers.

FY 2002 Accomplishments

- ICCVAM's evaluation of the Revised Up-and-Down Procedure (UDP) was published in two volumes (NIH Publication No. 02-4501) entitled, *The Revised Up-and-Down Procedure: A Test Method for Determining the Acute Oral Toxicity of Chemicals*. The revisions to the UDP include: (a) a modified up-and-down procedure with improved performance; (b) a modified Limit Test; and (c) a supplemental test for determining slope and confidence interval. The revised UDP has been proposed as an alternative to the existing conventional LD50 test.

- ICCVAM published its *Guidance Document on Using In Vitro Data to Estimate In Vivo Starting Doses for Acute Toxicity* at the end of FY '01/beginning of FY '02 (NIH Publication No. 01-4500). This guidance document was based upon the recommendations derived from the International Workshop on *In Vitro* Methods for Assessing Acute Toxicology, held October 2000 and organized by ICCVAM and the NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM).

- A Training Workshop on Acute Toxicity Testing Methods, organized by ICCVAM in partnership with EPA and International Life Sciences Institute (ILSI), was held February 2002. The workshop provided practical information and case studies to aid the understanding and use of the UDP and other *in vivo* and *in vitro* alternative methods for acute toxicity.

- ICCVAM has evaluated three alternative *in vitro* test methods for assessing the dermal corrosivity potential of chemicals and has published a report (NIH Publication No. 02-4502) entitled, *ICCVAM Evaluation of EPISKIN™, EpiDerm™ (EPI-200), and the Rat Skin Transcutaneous Electrical Resistance (TER) Assay: In Vitro Test Methods for Assessing the Dermal Corrosivity Potential of Chemicals*. ICCVAM concluded that these tests could be used as part of an integrated testing strategy for corrosivity/irritation, thereby reducing and refining the use of animals.

- To foster international collaborations in the alternatives arena, representatives of ICCVAM were invited to participate in the ECVAM (European Centre for the Validation of Alternative Methods) Status Seminar 2002 (June 2002) and the June 2002 meeting of the ECVAM Scientific Advisory Committee (ESAC), Ispra, Italy.

- ICCVAM's major input into and participation in the International Council for Laboratory Animal Science/Canadian Council for Animal Care (ICLAS/CCAC) International Symposium on Regulatory Testing and Animal Welfare (held June 2001) has resulted in the publication of a supplemental issue of the ILAR (Institute for Laboratory Animal Research)

Journal, Volume 43, 2002 containing the proceedings of that symposium, which include several publications contributed by ICCVAM members.

- ICCVAM has initiated its evaluation of the status of several *in vitro* assays proposed for use in the U.S. EPA's Endocrine Disruptor Screening Program (EDSP), i.e., estrogen and androgen receptor binding and transcriptional activation assays. Draft Background Review Documents (four volumes, April 2002) have been prepared which will serve as the starting point for the ICCVAM review.

- ICCVAM prompted the occurrence of, helped organize, and participated in an *OECD Conference on Validation and Regulatory Acceptance of New and Updated Methods in Hazard Assessment*, held March 2002. Several ICCVAM members were invited to make formal presentations, serve as discussion leaders, and to chair or serve as rapporteurs of various breakout groups.

- The ICCVAM website continues to evolve and is updated on a regular basis. It serves as a vital source of information related to ICCVAM activities, membership, test method evaluations, test methods under review, documents, publications, relevant guidelines/guidances/regulations, meetings, announcements, SACATM, etc. The URL for the website is: http://iccvam.niehs.nih.gov.

Center for Hepatotoxicity

The mission of the Center for Hepatotoxicity is to provide expertise in liver toxicology to the FDA. The focus of this group is two-fold and reflects the expertise of its members in the mechanistic analysis of toxic responses of the liver and in liver carcinogenesis.

The vision for the Center is to develop and apply an integrated biochemical, transcriptomic, proteomic and metabonomic approach to questions related to liver toxicology. Both cross-species and cross-cell type within the liver analyses will be performed. Biomarker profiles of liver toxicity will be generated for more effective assessment for risk of acute toxicity and of liver cancer development for application to individualization of therapy based on efficacy and safety profiles.

FY 2002 Accomplishments

In FY 2002, the Center for Hepatotoxicity was newly developed. The studies on the effect of chemical carcinogens on gene expression in human hepatocytes are near completion. Gender-specific gene expression profiles in the liver were developed. The liver toxicology of acetaminophen, tamoxifen, and the glitazones were examined by microarray and proteomic methods.

FY 2003 Plans

Staff will develop biomarkers for liver injury and disease, including hepatocarcinogenesis in mouse, rat, and human samples. The integration of signaling mechanisms for cell proliferation, apoptosis, and differentiation will provide a framework in which to assess these biomarkers.

Research Projects

Title	Project Number	Strategic Research Goal

PI: Dragan, Yvonne

- **Training in Hepatocyte Perfusion and Hepatic Cell Isolation** — **P00610** — **Predictive Toxicology**

  Objective(s):

  Train member(s) of the Hepatotoxicology Center in primary liver cell isolation and culture. The long-term goals will be to obtain signature gene and protein expression patterns of each cell type for comparison to toxin-induced changes. Training must be provided to give confidence in the integrity of liver cells following perfusion, separation, and culture of the liver cells.

PI: Harris, Angela

- **Modulation of Gene Expression in Chemical Carcinogenesis: Analysis of Aflatoxin B_1 Induced Gene Expression in Human Hepatocytes** — **E0704701** — **Concept-Driven**

  Objective(s):

  1) Verify aflatoxin B1 effects on steady-state mRNA levels of eight genes previously identified by differential hybridization of a gene filter array to be aflatoxin B1 (AFB_1)-responsive in human hepatocytes. Use Northern blot, RT-PCR and/or RNA protection assay to establish AFB_1 time and dose-response curves for maximal gene expression and also determine the minimum dose at which gene expression can be detected;
  2) Identify additional AFB_1-induced genes using differential display PCR (DD-PCR) and differential hybridization of a high-density filter array utilizing mRNA from human hepatocytes treated with low, moderate, and cytotoxic levels of AFB_1. Evaluate selected genes as described for objective #1;
  3) Distinguish genes involved in toxicological response to AFB_1 exposure from those that contribute to the carcinogenic response by comparing the gene expression profile of human hepatocytes treated with the hepatotoxic non-carcinogenic chemical, acetaminophen; and,
  4) Compare gene expression of selected genes in human hepatocytes treated with known rat liver chemical carcinogens, including 2-acetylaminofluorene, dimethylnitrosamine, and methapyrilene.

- **Development and Characterization of Conditionally Immortalized Human Primary Hepatocyte Cell Lines from Female and Male Donors** — **E0714101** — **Concept-Driven**

  Objective(s):

  Develop *in vitro* model systems for the study of mechanisms of toxicity in humans from different genders and/or ethnic populations.

Biochemical Toxicology

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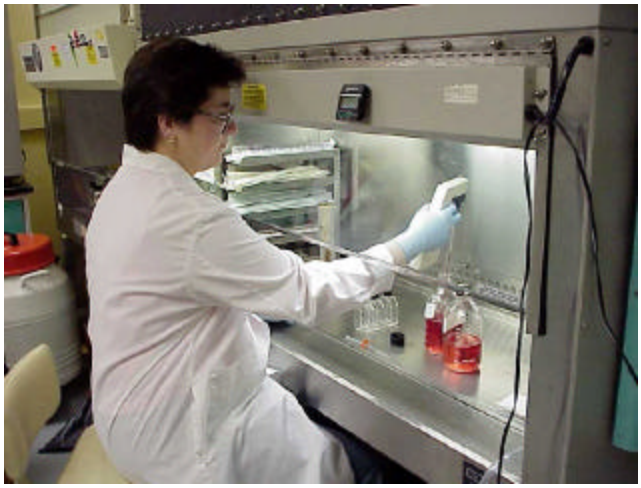

Marta Pogribna setting up cell culture for DNA methylation studies.

Executive Summary

Introduction

The Division of Biochemical Toxicology conducts fundamental and applied research specifically designed to define the biological mechanisms of action underlying the toxicity of products either regulated by or of interest to the Food and Drug Administration (FDA). This research centers on assessing the toxicities and carcinogenic risk associated with specific chemicals and gene-nutrient interactions, and the introduction of new techniques to assess toxicities and carcinogenic risk. The risk assessment research is firmly rooted in mechanistic studies focused on the understanding of toxicological endpoints, an approach that allows greater confidence in the subsequent carcinogenic risk assessments. Research within the Division capitalizes on scientific knowledge in the areas of biochemistry, organic chemistry, cellular and molecular biology, immunology, nutritional biochemistry, and pharmacology. It is supported by sound technical skills, the availability of state-of-the-art equipment, and internal and external collaborations and funding.

FY 2002 Accomplishments

A major emphasis within the Division is to conduct research on compounds nominated by the FDA for evaluation by the National Institute of Environmental Health Sciences, National Toxicology Program (NIEHS/NTP). This focus reflects the fact that the NCTR has superb animal facilities supported by a multi-disciplinary staff of scientists with strong mechanistic research experience; as such, the Center has the capability to conduct subchronic and chronic toxicological assessments in a rigorous manner to address the FDA's needs. While acknowledging the limitations of animal bioassays, these studies currently serve as the benchmark by which toxicological assessments are made by federal agencies, including the FDA. In addition to providing basic information on toxicological endpoints, such as cancer, these experiments form the basis for mechanistic studies to ascertain if the response detected in the experimental model is pertinent to humans.

During FY 2002, division investigators completed NTP studies to determine the effect of ethanol upon the carcinogenicity of urethane, a fermentation product found in certain foods. The results indicated that ethanol potentiated or attenuated the carcinogenicity of urethane depending upon

the sex of the test animal and the tissue being examined. These data will aid the FDA Center for Food Safety and Applied Nutrition (CFSAN) to set regulatory levels for this natural contaminant. The carcinogenicity of malachite green, a therapeutic agent used in aquaculture, was also investigated in response to an NTP nomination by the FDA Center for Veterinary Medicine (CVM). Although these studies are not complete, preliminary data indicate that leucomalachite green, a metabolite of malachite green, is a mouse liver carcinogen. Division investigators also elucidated the mechanism by which riddelliine, a pyrrolizidine alkaloid found in herbal teas, is activated to a genotoxic carcinogen.

An area of particular concern to the FDA, in particular CFSAN, is the potential toxicity of cosmetic ingredients due to their interaction with light. To address this concern, the NCTR in collaboration with the NIEHS/NTP constructed a phototoxicology facility that is located within the Division. Initial studies at the NCTR Center for Phototoxicology focused on the co-carcinogenic effects of simulated solar light and topically applied a- and ß-hydroxy acids, and during the year were expanded to include similarly designed studies with topically applied *Aloe vera* and retinyl palmitate. More recently, in collaboration with scientists at CFSAN, experiments were initiated to quantify the chemicals used in tattoo inks and to investigate the photostability and safety of pigments used for tattooing, including permanent make-up. Other experiments focused on determining the changes in gene expression associated with 8-methoxypsoralen/UVA (PUVA) therapy and on developing a transgenic mouse model to investigate the mechanisms for the induction of cutaneous malignant melanoma.

As part of the NTP effort, the Division investigators studied a series of endocrine-active compounds, including genistein, ethinyl estradiol, nonylphenol, and vinclozolin. These studies, which are still ongoing, involve both short-term and long-term assessments that are unique in terms of the number of endpoints evaluated and the inclusion of chronic toxicity assessments using varied exposure windows.

Anti-retroviral drugs are being used to prevent the mother-to-child transmission of human immunodeficiency virus type 1, the virus responsible for acquired immunodeficiency syndrome. While effective in preventing viral transmission, the long-term consequences of perinatal exposure to these drugs are presently unknown. Division investigators conducted a series of investigations to examine the genotoxic consequences of anti-retroviral nucleoside analogues in neonatal mice. Initial experiments indicated that zidovudine, but not lamivudine, is mutagenic, and that lamivudine does not alter the responses induced by zidovudine. During the year these studies were expanded to include the nucleoside analogues stavudine, didanosine, and zalcitabine, and the non-nucleoside reverse transcriptase inhibitor nevirapine.

Tamoxifen is an adjunct chemotherapeutic agent for the treatment of breast cancer and a chemoprotective agent for breast cancer prevention. Despite being beneficial in regard to breast cancer, tamoxifen is known to increase the risk of endometrial cancer in women. Division investigators conducted experiments to elucidate the mechanisms for tumor induction, with emphasis on characterizing the DNA adducts formed from this drug. As part of this effort, mass spectral methods were developed with sufficient sensitivity to detect and quantify tamoxifen DNA adducts in women receiving the drug. Similar mass spectral methods were also developed for DNA adducts arising from 4-aminobiphenyl and urethane, as well as oxidative DNA damage.

Other investigations focused in understanding the metabolic pathways associated with isoflavones found in various dietary supplements.

A strong emphasis within the Division has been in the area of nutritional folic acid deficiency and tumor progression. As part of this program, Division investigators evaluated the progressive changes in global DNA hypomethylation and promoter region hypermethylation in the p16 tumor suppressor gene using a rat model of hepatocellular carcinogenesis. They also developed a profile of plasma thiol metabolites associated with folate-dependent homocysteine metabolism that can be used to determine the functional significance of relevant genetic polymorphisms and homocysteine-related pathology in humans.

FY 2003 Plans

In FY 2003, the Division's NTP efforts will include completion of final reports on the carcinogenicity of a- and ß-hydroxy acids and malachite green. Toxicity reports will also be prepared for genistein, ethinyl estradiol, nonylphenol, and vinclozolin. Photocarcinogenicity and mechanistic studies will be conducted on topically applied *Aloe vera* and retinyl palmitate, and the subchronic toxicity of *Aloe vera* will be assessed following oral exposure. Multigeneration studies will continue with the endocrine-active compounds, and studies will be initiated to assess the effects of perinatal exposure of zidovudine and lamivudine in combination with the non-nucleoside reverse transcriptase inhibitor nevirapine and the protease inhibitor nelfinavir. At the request of CFSAN, experiments will be initiated on acrylamide, a carcinogen found in fried foods. These experiments will emphasize dose-response relationships and the development of biomarkers for assessing exposure.

In addition to conducting bioassays on *Aloe vera* and retinyl palmitate, investigators associated with the NCTR Center for Phototoxicology will continue investigations on the interaction of light with tattoo pigments. They will also begin experiments to establish the optimal conditions for housing SKH-1 hairless mice, determine gene expression in response to photocarcinogens, and continue investigations on transgenic mouse models for photocarcinogenesis, with emphasis on the induction of cutaneous and ocular melanoma.

Results from the studies with endocrine-active compounds have indicated that soy-containing diets may protect against renal toxicity. Gene and protein expression will be used to elucidate the mechanisms behind this effect. In addition, the potential protective effects of endocrine-active compounds upon bone density will be investigated.

Experiments with tamoxifen will be expanded to determine if tamoxifen-DNA adducts can be detected in target tissues of women receiving the drug. These investigations will take advantage of recently developed methodologies including HPLC/mass spectrometry and chemi-luminescence immunoassay. Mechanistic investigations will continue to elucidate the metabolic pathways on isoflavones and experiments will be initiated to investigate the potential genotoxicity of components found in hormone replacement therapies. Experiments with pyrrolizidine alkaloids will be expanded to establish if the mechanisms of metabolic activation for riddelliine are applicable to other pyrrolizidine alkaloids.

Research Projects

Title	Project Number	Strategic Research Goal

PI: Beland, Frederick

- **Effect of Ethanol on the Tumorigenicity of Urethane (Ethyl Carbamate) in B6C3F1 Mice** — **E0212001** — **Agent-Driven**

  Objective(s):

  Determine the effect of ethanol on the tumorigenicity of urethane (ethyl carbamate) in B6C3F1 mice.

- **Perinatal Carcinogenicity of Drug Combinations used to Prevent Mother-to-Child Transmission of HIV** — **E0214111** — **Agent-Driven**

  Objective(s):

  Determine the carcinogenicity, genotoxicity, and metabolism of antiretroviral drug combinations administered to mice transplacentally, perinatally, or neonatally.

- **DNA Adducts of Tamoxifen** — **E0701101** — **Agent-Driven**

  Objective(s):

  The nonsteroidal antiestrogen tamoxifen, which is currently being used in clinical trials as a chemoprotective agent against breast cancer, has been associated with the induction of certain malignancies. In order to determine if tamoxifen is acting through a genotoxic mechanism, this project will characterize DNA adducts from suspected tamoxifen metabolites, and develop methods for their detection and quantitation.

- **Salmonella Mutagenicity Testing for Regulatory Needs** — **S00179** — **Concept-Driven**

  Objective(s):

  Use the Ames Salmonella mutagenicity test system to determine the mutagenicity of compounds of regulatory interest to the Agency.

- ***In vivo* DNA Adduct Standards** — **S00198** — **Concept-Driven**

  Objective(s):

  This Support number is being set up to take the place of P00371 - Per division, this will be an ongoing support number in collaboration with IARC. - P00371 has been closed out.

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

PI: Boudreau, Mary

- **Effects of *Aloe Vera* Components on Cell Proliferation and DNA Adduct Formation in SKH-1 Mice Following Simulated Solar Light Exposure** — E0214001 — Agent-Driven

  Objective(s):

  1) Determine the dose response and acute kinetics of topical exposure to *Aloe vera* plant components on the structure of SKH-1 mouse skin in the absence of simulated solar light exposure;
  2) Determine the effects of topical exposure of *Aloe vera* plant components on the amount of simulated solar light required to induce skin edema in the SKH-1 mouse;
  3) Determine the subchronic effects of repeated co-exposure to *Aloe vera* plant components and simulated solar light on skin cell edema, proliferation, and DNA damage in the SKH-1 mouse;
  4) Determine the tumor-promoting activities of *Aloe vera* plant components following simulated solar light tumor initiation; and,
  5) Determine the influence of *Aloe vera* components on simulated solar light-induced tumor formations in mice.

- **Bioassays in the F-344 Rat and the B6C3F1 Mouse Administered *Aloe Vera* Plant Constituents in the Drinking Water** — E0214201 — Agent-Driven

  Objective(s):

  The use of *Aloe vera* is not limited to over-the-counter dermal therapeutics and cosmetics. *Aloe vera* is also taken internally, and *Aloe vera* for internal consumption is also widely used as a prophylaxis and treatment for a variety of unrelated systemic conditions. In view of the complexities inherent in aloe pharmacology and the inconsistencies reported in literature, the objective of these studies is to conduct bioassays in rats and mice using standardized preparations of *Aloe vera* to explore the limits of safety for the *Aloe vera* leaf constituents present in commercial products.

Title	Project Number	Strategic Research Goal

PI: Chou, Ming

- **A Study of Genotoxic and Secondary Mechanisms of Riddelliine Carcinogenesis** — **E0213301** — **Predictive Toxicology**

  Objective(s):

  1) Study the mechanisms of direct-acting genotoxicity (involving exogenous DNA adduct formation) of riddelliine;
  2) Analyze riddelliine-derived DNA adducts in target tissues of rats treated with riddelliine as part of the NTP chronic study, and from male and female rats to be treated at the NCTR for a shorter period of time with riddelliine and its reactive metabolite, dehydroriddelliine;
  3) If a dehydroretronecine-modified DNA adduct is detected in the liver tissues of animals treated with riddelliine, propose to determine whether or not this DNA adduct is also formed in animals treated with other tumorigenic pyrrolizidine alkaloids; and,
  4) Compare the metabolic activation pathways and DNA adduct formation of the tumorigenic pyrrolizidine alkaloid, riddelliine, retrorsine, and monocrotaline, and a non-tumorigenic pyrrolizidine alkaloid, retronesine in rat and human liver microsomal systems.

- **A Collaborative Research Proposal to Assess Cancer Risk Posed by Intermittent Exposure to Aflatoxin B1 in Rats** — **E0688801** — **Agent-Driven**

  Objective(s):

  1) Test the hypothesis that a chemically induced tumor incidence is a function of the accumulated lifetime exposure, and is predictable from the average daily dose for various dosing regimens, such as continuous and intermittent dosing; and,
  2) Study correlations between the chemically induced tumor incidence and various biomarkers of the initiation and the promotion stage of carcinogenesis for continuous and intermittent dosing.

Title	Project Number	Strategic Research Goal

- **Effects of Dietary Restriction on the Post-Initiation Stages in Aflatoxin AFB1-Induced Carcinogenesis on Male F-344 Rats Fed Methyl-Deficient Diets** — E0695201 — Concept-Driven

Objective(s):

Study the interactions of dietary restriction (DR) and methyl deficiency (MD) on the alterations of hepatic oxidative DNA damages, DNA methylation, cell proliferation, oncogene and tumor suppressor gene mutation, preneoplastic foci formation and tumor incidence during the post-initiation stages of AFB1-induced carcinogenesis in male F344 rats. The results of these studies will: (i) test the hypothesis that DR may be an antagonist to the promotional effect of MD in the AFB1-induced carcinogenesis; and (ii) evaluate the correlations between the effects of DR and MD on the formation of AFB1-induced preneoplastic foci and tumors and various biomarkers during the post-initiation stages of carcinogenesis.

Title	Project Number	Strategic Research Goal

- **A Study of Genotoxic Mechanisms of Carcinogenic Pyrrolizidine Alkaloids and Pyrrolizidine Alkaloid N-Oxides** — **E0710401** — **Predictive Toxicology**

Objective(s):

1) Characterize the structures of the eight dehydroretronecine (DHP)-derived DNA adducts;
2) Study metabolism of retronecine-based pyrrolizidine alkaloids, heliotridine-based pyrrolizidine alkaloids, otonecine-based pyrrolizidine alkaloids, and pyrrolizidine alkaloid N-oxides by liver microsomes of F344 rats, $B6C3F_1$, mice, and humans of both sexes, and compare metabolism profiles;
3) Study the DNA adduct formation *in vitro* (from liver microsomal metabolism of the pyrrolizidine alkaloids described above in the presence of calf thymus DNA) and *in vivo,* and determine whether or not the same set of DHP-derived DNA adducts is formed in all cases;
4) Determine whether or not the levels of DHP-derived DNA adducts from different types of necine-based pyrrolizidine alkaloids formed in target tissues (liver) are significantly higher than those in non-target tissues;
5) Determine whether or not pyrrolizidine alkaloid N-oxides can be metabolized by rat and mouse liver microsomes to the parent pyrrolizidine alkaloids and whether or not DHP-derived DNA adducts are formed in significant amounts both *in vivo* and *in vitro*;
6) Determine whether or not some dietary supplements sold in the United States contain genotoxic pyrrolizidine alkaloids;
7) Determine the effect of liver carboxyesterases on DHP-derived DNA adduct formation from rat and human liver microsomal metabolism in the presence of calf thymus DNA;
8) Determine the effect of liver carboxyesterase inhibitors on DHP-derived DNA adduct formation from rat and human liver microsomal metabolism in the presence of calf thymus DNA; and,
9) Determine the effect of Chinese herbs, such as liquorice, and their active components, such as glycyrrhizin and glycyrrhetinic acid, on inhibition of DHP-derived DNA adduct formation *in vivo* and *in vitro*.

PI: Culp, Sandra

- **Two-Year Bioassay in Mice Administered Malachite Green or Leucomalachite Green in the Diet** — **E0212701** — **Agent-Driven**

Objective(s):

Determine the risk associated with exposure to malachite green or leucomalachite green.

Title	Project Number	Strategic Research Goal

- **Two-year Bioassay in Rats Administered Malachite Green or Leucomalachite Green in the Diet** — **E0212801** — **Agent-Driven**

  Objective(s):

  Determine the risk associated with exposure to malachite green or leucomalachite green.

PI: Delclos, Kenneth

- **Range Finding Study for the Evaluation of the Toxicity of Genistein Administered in the Feed to CD (Sprague-Dawley) Rats (Without Behavioral Breeding)** — **E0212201** — **Agent-Driven**

  Objective(s):

  Determine the doses of genistein to be used in a multigeneration bioassay for establishing the effects of this naturally occurring isoflavone on development of reproductive organs, reproduction, cancer of the reproductive organs, and neurological and immunological function.

- **Range Finding Study for the Evaluation of the Toxicity of Nonylphenol Administered in the Feed to CD (Sprague-Dawley) Rats** — **E0212501** — **Agent-Driven**

  Objective(s):

  Determine the doses of nonylphenol for use in a multigeneration bioassay for assessing the effects of this compound on the development of the reproductive tract, reproduction, and neurological and immunological function.

- **Range Finding Study for the Evaluation of the Toxicity of Vinclozolin Administered in the Feed to CD (Sprague-Dawley) Rats** — **E0212601** — **Agent-Driven**

  Objective(s):

  Determine the doses of vinclozolin for use in a multigeneration bioassay for assessing the effects of this compound on the development of the reproductive tract, reproduction, and neurological and immunological function.

- **Range Finding Study for the Evaluation of the Effects of Ethinyl Estradiol Administered in the Feed to CD (Sprague-Dawley) Rats During Development** — **E0212901** — **Agent-Driven**

  Objective(s):

  Determine the doses of ethinyl estradiol (EE2) for use in a multigeneration bioassay for assessing the effects of this compound on the development of the reproductive tract, reproduction, and neurological and immunological function.

Title	Project Number	Strategic Research Goal

- **A Comparison of Weight Gain and Fertility in CD Rats Fed a Standard Diet (NIH-31) or a Soy- and Alfalfa-free, Casein-containing Diet (NIH-31C)** — E0213001 — Agent-Driven

  Objective(s):

  Evaluate effects of NIH-31C on fertility by comparing pregnancy rates and litter size and weight in CD rats treated according to the treatment regimen to be used in the F0 generation of the multigeneration.

- **Genistein: Evaluation of Reproductive Effects Over Multiple Generations and the Chronic Effects of Exposure during Various Life Stages** — E0213201 — Agent-Driven

  Objective(s):

  1) Determine the effects of genistein, a naturally occurring isoflavone, on reproduction and on the development of reproductive and selected other hormone-sensitive organs when administered to CD rats over multiple generations;
  2) Determine if subtle effects observed in the dose range finding study are magnified through multiple generations;
  3) Evaluate the reversibility of any observed effects; and,
  4) Evaluate the chronic toxicity of genistein, particularly potential induction of cancer of the reproductive organs, following exposures that will include various life stages (*in utero* through early adulthood, *in utero* and continuous for two years after birth, *in utero* and lactational only, and postweaning only).

- **para-Nonylphenol: Evaluation of Reproductive Effects over Multiple Generations** — E0213501 — Agent-Driven

  Objective(s):

  1) Determine the effects of p-nonylphenol, an intermediate in the production of surfactants and other industrial products, on reproduction and on the development of reproductive and selected other hormone-sensitive organs when administered to CD rats over multiple generations;
  2) Determine if subtle effects observed in the dose range finding study are magnified through multiple generations; and,
  3) Evaluate the reversibility of any observed effects.

Title	Project Number	Strategic Research Goal

- **Ethinyl Estradiol: Evaluation of Reproductive Effects over Multiple Generations and the Chronic Effects of Exposure during Various Life Stages** — **E0213801** — **Agent-Driven**

  Objective(s):

  1) Evaluate the effects of ethinyl estradiol, a potent synthetic estrogen widely used in prescription drugs, on reproduction and on the development of reproductive and selected other hormone-sensitive organs when administered to CD rats in the diet over multiple generations;
  2) Determine if subtle effects observed in the dose range finding study are magnified through multiple generations;
  3) Evaluate the reversibility of any observed effects, and,
  4) Evaluate the chronic toxicity of ethinyl estradiol, particularly the potential induction of cancer of the reproductive organs, following exposures that will include various life stages.

- **Sexual Dimorphism in the Inflammatory Response to Biomaterials** — **E0696301** — **Agent-Driven**

  Objective(s):

  Determine if a sex difference in the *in vitro* response of human monocytes and mouse peritoneal macrophages to various biomaterials can be demonstrated. Based on existing literature, we hypothesize that there will be a significant sex difference in the synthesis and release of inflammatory mediators that could influence the biocompatibility of the material.

- **The Effects of Dietary Genistein on the Growth of Chemically Induced Mammary Tumors in Ovariecto-mized and Intact Rats** — **E0702701** — **Agent-Driven**

  Objective(s):

  This proposal will determine whether or not, in the absence of endogenous ovarian estrogens, dietary genistein can promote or suppress the growth of neoplastic mammary tissue at various stages for the carcinogenic process.

- **Effects of Endocrine-Active Agents on Bone** — **E0710601** — **Agent-Driven**

  Objective(s):

  We hypothesize that the administration of the endocrine-active agents genistein and ethinyl estradiol (EE2) will alter bone growth and remodeling and that the direction and extent of the effect will depend on the window of exposure to the compounds. Utilize the experience of Bionetics staff and tissues available from the on-going endocrine disruptor studies to address an important health concern.

Title	Project Number	Strategic Research Goal

PI: Doerge, Daniel

- **Toxic Hazards from Anti-thyroid Chemicals** **E0692001** **Concept-Driven**

  Objective(s):

  1) Determine inhibition mechanisms for environmental goitrogens using purified thyroid peroxidase and lactoperoxidase;
  2) Determine the mechanism for covalent binding suicide substrates to purified peroxidases using electrospray-mass spectrometry to analyze intact adducted proteins and/or proteolytic fragments;
  3) Determine mechanism of goitrogen uptake into isolated thyroid cells in primary culture and subsequent inhibition of iodination/coupling reactions involved in thyroid hormone synthesis; and,
  4) Determine the structure-activity relationship for uptake of goitrogens into the thyroid and inhibition of thyroid-hormone synthesis rats.

- **Development of Methods for Analysis and Confirmation of ß-Agonists** **E0694501** **Method-Driven**

  Objective(s):

  1) Develop determinative and confirmatory procedures using Liquid Chromatography-Atmospheric Pressure Chemical Ionization Mass Spectrometry (LC-APCI/MS) for multiresidue screening ß-agonists in livestock tissues;
  2) Develop synthetic procedures to produce authentic ß-agonist standards for use in regulatory screening. These methods will provide the flexibility to adapt to the potential for "designer drug" modifications by clandestine laboratories; and,
  3) Explore the use of packed column supercritical fluid chromatography (SFC) coupled to APCI/MS as a more efficient technique for chromatographic separation in the screening of large numbers of ß-agonists in livestock issues.

- **Measurement of Oxidative DNA Damage in Normal and Hepatitis C-Infected Human Liver** **E0706401** **Method-Driven**

  Objective(s):

  1) Develop simple synthetic methods to produce stable labeled analogs of 8-oxo-dG, etheno-dA, etheno-dC, and M1-dG;
  2) Develop an automated on-line sample preparation method to maximize detection sensitivity for 8-oxo-dG, etheno-dA, etheno-dC, and M1-dG, in a single sample analysis, using liquid chromatography and tandem mass spectrometry; and,
  3) Apply methodology to the analysis of hepatic DNA from humans and animals; and
  4) Determine feasibility for application to clinical trials of therapeutic agents and toxicity/carcinogenicity testing in experimental animals.

Title	Project Number	Strategic Research Goal

- **Human Studies of Isoflavone Safety and Efficacy** **S00607** **Method-Driven**

  Objective(s):

  Bioanalytical analysis of soy isoflavones (and metabolites) in support of clinical trials at the University of Miami and Wayne State University.

PI: Fu, Peter

- **Effect of Topically Applied Skin Creams Containing Retinyl Palmitate on the Photocarcinogenicity of Simulated Solar Light in SKH-1 Mice** **E0214301** **Predictive Toxicology**

  Objective(s):

  Study the effects of topically applied skin cream containing Retinyl Palmitate on the photocarcinogenicity of simulated solar light in SKH-1 mice.

- **The Evaluation of Selected Benzodiazepine and Antihistamine Drugs in the Neonatal Mouse Tumorigenicity Bioassay and in Transgenic Human Lymphoblastoid Cells** **E0687901** **Predictive Toxicology**

  Objective(s):

  1) Determine if the neonatal mouse bioassay can be employed to evaluate the tumorigenic potential of therapeutic drugs;
  2) Examine concurrently as positive controls the genotoxic carcinogens: 4-aminobiphenyl, benzo(a)pyrene, 6-nitrochrysene, and aflatoxin B1;
  3) Study the metabolism and DNA adduct formation of benzodiazepine and antihistamine drugs by mouse and human liver microsomes to determine which, if any, cytochrome P450 is responsible for metabolic activation in mice and humans; and,
  4) Employ transgenic human lymphoblastoid cell lines expressing appropriate CYP isozymes to study the mutations and DNA binding of the subject drugs.

Title	Project Number	Strategic Research Goal

- **A Study of the Secondary Mechanisms of Carcinogenesis: Lipid Peroxidiation and Endogenous DNA Adduct Formation from Chloral Hydrate, Benzodiazepines, Antihistamines, and Other Chemicals** — E0700401 — Predictive Toxicology

  Objective(s):

  The specific aims outlined below are critical for the development of methodologies to study secondary mechanisms of carcinogenesis, including lipid peroxidation and endogenous DNA adduct formation, for determination of the mechanisms by which chemicals, such as FDA-regulated drugs including benzodiazepines and antihistamines, may induce cancer, and for the continued development of the neonatal mouse bioassay as a regulatory alternative tumorigenicity bioassay to:

  1) Develop analytical methodologies for analysis of lipid peroxidation products and endogenous DNA adducts;
  2) Determine whether or not the drugs of FDA interest, including benzodiazepines and antihistamines studied in E687901, and other chemicals, induce lipid peroxidation and endogenous DNA adduct formation *in vitro*;
  3) Determine the inhibitory effect of lipid- and water-soluble antioxidants on drug-induced lipid peroxidation and endogenous DNA adduct formation *in vitro*;
  4) Determine whether or not the malondialdehyde-modified MG-1 DNA adduct and/or other endogenous DNA adducts can be used as biomarkers of lipid peroxidation; and,
  5) Determine the mutagenicity of the benzodiazepine and antihistamine drugs in *Salmonella typhimurium* TA104 and determine whether or not mutagenicity in *Salmonella typhimurium* TA104 can be used as a biomarker of lipid peroxidation induced by chemicals that generate free radicals upon metabolism.

PI: Howard, Paul

- **Chronic Tumor Study of Fumonisin B1 in Male and Female B6C3F1 Mice** — E0210601 — Agent-Driven

  Objective(s):

  Determine if dietary fumonisin B1 is tumorigenic to male and female B6C3F1 mice following chronic dietary exposure.

- **Chronic Tumor Study of Fumonisin B1 in Male and Female F344 Rats** — E0210801 — Agent-Driven

  Objective(s):

  Determine the tumorigenicity of fumonisin B1 in male and female F344 rats following chronic dietary exposure.

Title	Project Number	Strategic Research Goal

- **The Role of Fumonisin B1 in Fusarium sp. Tumorigenicity in Rats** — E0211101 — Agent-Driven

  Objective(s):

  Determine the effect of fumonisin B1 on signal transduction pathways in cultured human esophageal epithelial tissues. Determine if DNA damage occurs *in vivo* in F344 rats when fed in the diet cultures of *Fusarium graminearum, Fusarium subglutinans, Fusarium moniliforme* or a combination of the three fungi, using 32P-postlabeling technique. Determine the pharmacokinetics of fumonisin B1 in B6C3F1 mice and F344 rats under conditions similar to those used in the chronic bioassay, and in non-human primates.

- **Comparative Toxicity of Fumonisin Derivatives in Female B6C3F1 Mice** — E0212401 — Agent-Driven

  Objective(s):

  The primary objective of the study is to compare the toxicity of several fumonisin derivatives in female B6C3F1 mice.

- **The Effects of Chemoexfoliation using a- and ß-hydroxy Acids on Cell Proliferation and DNA Adduct Formation in SKH-1 Mice Exposed to Simulated Solar Light** — E0213101 — Agent-Driven

  Objective(s):

  The NIEHS/FDA Phototoxicology Center is designed to address the effects of compounds on the induction of skin cancer in mice using light sources that are relevant to humans. Input into the design of the facility has been obtained from experts in phototoxicity and photocarcinogenicity. These experts will continue to provide critical advice on the design of the experimental protocols. As a result, a facility has been developed that will meet the rigors of scientific scrutiny, and will generate data for human health risks from the effects of compounds on light-induced skin cancer. The facility is also designed for expansion to allow simultaneous examination of the toxicity or cocarcinogenicity of compounds in the presence of either simulated sunlight or fluorescent UVB light. The mechanistic studies in this proposal will provide the data necessary to design and interpret properly the future a-hydroxy acid and simulated solar light cocarcinogenicity studies.

  Objective(s):

  Detailed followup studies to address results of some of the experiments outlined in E0213111 and to experimentally address changes that were incorporated into the AHA/BHA chronic study (E0213701). Repeats of exp. 8.1.6, additional immunohistochemistry on exp. 8.3.2.3,

Title	Project Number	Strategic Research Goal

- **Effect of Topically Applied Skin Creams Containing Glycolic and Salicylic Acid on the Photocarcinogenicity of Simulated Solar Light in SKH-1 Mice** — **E0213701** — **Agent-Driven**

  Objective(s):

  Determine if the application of creams containing a- and ß-hydroxy acids to the skin of male and female SKH-1 hairless mice alters the tumor incidence induced by simulated solar light in the mouse skin.

- **The Use of DNA Microarray Technology to Quantify the Effects of 8-methoxypsoralen (8-MOP) and UVA Light Treatment on SKH-1 Mouse Skin** — **E0213901** — **Agent-Driven**

  Objective(s):

  Determine the effects of PUVA treatment on gene expression in the skin of SKH-1 mice. Success of this project will lead to a more extensive protocol in collaboration with NIEHS.

- **DNA Adduct Formation by Nicotine Metabolites** — **E0692501** — **Concept-Driven**

  Objective(s):

  1) Determine the structural identity of the nicotine delta 1',2'- and delta 1',5'-iminium ion DNA adducts, and modify existing 32P-post labelling techniques to detect the adduct; and,
  2) Quantify the presence of these adducts *in vitro* and *in vivo* in mice.

- **Purification of Ceramide Synthase** — **E0705901** — **Concept-Driven**

  Objective(s):

  1) Isolate rat ceramide synthase;
  2) Identify the gene coding for rat ceramide synthase;
  3) Develop antibodies to rat ceramide synthase; and,
  4) Use the antibodies to study tissue-specific expression of ceramide synthase

Title	Project Number	Strategic Research Goal

- **Methodology for Safety Testing of Pigments Used for Tattooing, Including Permanent Make-up** — E0710501 — Method-Driven

  Objective(s):

  1) Determine the chemicals in tattoo pigments and their metabolism *in vitro*;
  2) Develop methodology for tattooing SKH-1 hairless mice in a quantitative and reproducible manner;
  3) Determine the extent of inflammation induced by the implanted pigment, and determine the time of recovery following tattooing;
  4) Determine the acute toxicity of several tattoo inks and permanent make-up inks in SKH-1 hairless mice in the presence and absence of simulated solar light; and,
  5) Determine if tattoo pigments are photococarcinogenic in the SKH-1 hairless mouse using simulated solar light.

- **Historical Database of Skin Tumor Formation in SKH-1 Mice** — S00213 — Knowledge Base

  Objective(s):

  1) Enter into the NCTR MultiGen system the historical data (Argus Research Laboratories) of the tumor incidence in SKH-1 mice treated with simulated solar light (SSL) (no test compounds). Argus Research Laboratories will provide the weekly individual animal observation records for as many studies as deemed reasonable by Argus Research Laboratories. NCTR will be responsible for entering the data into the NCTR MultiGen database;
  2) NCTR will generate tumor incidence reports summarizing the occurrence of tumors in the animal groups from the animal tumor data that were obtained from Argus Research Laboratories. The data will then be analyzed for various parameters pertinent to these types of studies (e.g., time to first tumor, mean time to first tumor, tumors/mouse). The data, summary data, and statistical analyses will be shared with Argus Research Laboratories;
  3) NCTR will share with Argus Research Laboratories the incidence data on the occurrence of tumors in control SKH-1 mice treated with SSL at NCTR. NCTR will additionally share with Argus Research Laboratories the development of any statistical methods for analyzing the tumor incidence data with SKH-1 mice; and
  4) Both parties agree to share information concerning future development of: animal rack and caging systems; devices for holding the SSL lamps; control of SSL lamps; devices for housing fluorescent light fixtures; devices, software, or protocol for monitoring the irradiance from SSL or fluorescent lights; and database systems for collecting animal information.

Title	Project Number	Strategic Research Goal

PI: James Gaylor, Sandra

- **Nutritional Modulation of Apoptosis and Chemosensitivity: A Novel Anticancer Strategy** — **E0700301** — **Concept-Driven**

  Objective(s):

  1) In Nitroso methylurea (NMU)-initiated mammary epithelial cells, determine whether nutritional manipulation of the cell cycle combined with low-dose chemotherapy will permanently eliminate p53-independent and p53-dependent preneoplastic and neoplastic cells; and,
  2) Determine the mechanisms of cell death induced by nutritional manipulation and low-dose chemotherapy by examining molecular endpoints associated with p53-dependent and independent pathways of apoptosis.

- **Molecular and Metabolic Determinants of Maternal Risk and Progression of Down Syndrome: Potential for Nutritional Interventions** — **E0701601** — **Concept-Driven**

  Objective(s):

  1) Define abnormalities in one-carbon metabolism in mitogen-stimulated lymphocytes from women who have had a child with Down Syndrome, and determine whether appropriate folate/methyl supplementation can normalize these metabolic abnormalities; and,
  2) Define the biochemical and molecular consequences of abnormal one-carbon metabolism in mitogen-stimulated lymphocytes from Down Syndrome children, and determine whether these metabolic abnormalities can be normalized with targeted nutritional intervention.

- **DNA Damage with Dietary Methyl Donor Deficiency** — **E0706501** — **Concept-Driven**

  Objective(s):

  Further the understanding of the mechanisms by which diet, as an environmental variable, can alter the susceptibility to cancer.

- **Genes, Micronutrients, and Homeobox-related Mal-formations** — **E0707201** — **Predictive Toxicology**

  Objective(s):

  A more specific understanding of the genetic and environmental factors that contribute to the etiology of birth defects is a necessary prerequisite for the design of effective preventive strategies to reduce infant and maternal risk. This project has the potential to significantly advance current knowledge of specific etiological factors involved in maternal and infant risk, to aid in the design of nutritional intervention strategies, and to provide a basis for future mechanistic studies of human malformation. NCTR's main objective is investigation of maternal risk factor for neural tube defects and congenital heart defects.

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Title	Project Number	Strategic Research Goal

- **Folic Acid Metabolism in Children with Down Syndrome (DS)** **E0708501** **Concept-Driven**

  Objective(s):

  Determine whether supplementation with the nutrients folinic acid and betaine will increase plasma levels of methionine, S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH), which have shown to be low in children with DS. The experiments will focus on the biochemical lesions in one-carbon metabolism stemming from trisomy 21 gene overdose and the potential to normalize metabolic imbalance with targeted nutritional intervention. With better understanding of the metabolic and molecular aberrations of cystathionine beta synthase (CBS) gene overdose in DS, the potential to ameliorate or prevent these progressive disease processes with nutritional intervention could become a reality. In the proposed study, baseline levels of homocysteine, methionine, cystathionine, cysteine, glutathione, cysteinyl-glycine, SAM, SAH, and adenosine in plasma of Down Syndrome children will be determined at baseline and after 3 months supplementation with folinic acid and betaine. This will define the metabolic abnormalities in one-carbon metabolism caused by the presence of an extra copy of chromosome 21 and is an important first step in determining whether there is a potential for nutritional intervention to correct the metabolic imbalance. The long-term goal for this study is to determine whether nutritional intervention in children with DS at 2-10 years of age will have a positive effect on their growth, immunologic function, and cognitive development. Adults with DS have already reached a plateau of growth and development and therefore the likelihood that nutritional intervention will affect their growth and development is minimal.

Title	Project Number	Strategic Research Goal

- **Mechanisms and Consequences of DNA Damage and Methylation Dysregulation during Rat Hepato-carcinogenesis** — E0712801 — Concept-Driven

Objective(s):

1) Confirm that the presence of uracil and abasic sites in preneoplastic DNA from folate/methyl-deficient rats creates nonproductive high-affinity binding sites for the DNA methyltransferase that compromise normal DNA methylation at the replication fork resulting in genome-wide hypomethylation;
2) Determine whether the double-stranded loss of cytosine methylation is maintained in folate-/methyl-deficient rats after nutritional repletion of methyl donors or whether the original methylation pattern and chromatin structure can be reestablished; and whether the increase in dnmt1 expression is stimulated by global loss of methyl groups and whether dnmt1 expression is decreased by methyl repletion;
3) Determine the temporal relationship between the appearance of DNA lesions and site-specific methylation within the CpG island of the p16 promoter region in p16 gene expression with alterations in local chromatin structure and DNA methyltransferase mRNA levels and activity; and,
4) Use microarray slides printed with the rat cDNA library in collaboration with Dr. James Fuscoe as a tool to screen for methylation-related down-regulation of candidate genes in hepatic preneoplastic foci, preneoplastic nodules, and tumor tissue from folate-/methyl-deficient rats.

PI: Roberts, Dean

- **Antigenic Biomarkers of Estrogen Catechol Metabolism for Use in Epidemiological Studies** — E0705701 — Predictive Toxicology

Objective(s):

1) Prepare immunogenic conjugates for immunization of rabbits and antigenic conjugates for the characterization of antisera and for affinity purification of antibodies;
2) Develop immuno affinity/liquid chromatography/mass spectrometry (IA/LC/MS) methods to detect the antigenic biomarkers in urine and/or serum; and,
3) Initiate studies to validate the use of the antibody reagents and IA/LC/MS methods developed in Aim 1 and 2 using human urine and serum samples collected in an ongoing study of reproductive events, carcinogen metabolism, and interindividual variability.

Title	Project Number	Strategic Research Goal

PI: Tolleson, William

- **Molecular Basis of Tumor Promotion and Increased Somatic Growth in Yellow Avy/a Mice: Mitogenic Effects of Agouti Protein *in vitro*** — **E0701201** — **Concept-Driven**

  Objective(s):

  Determine whether or not the agouti protein stimulates mitogenesis *in vitro*.

- **The Role of Human Metabolism in Endocrine Disruption** — **E0702301** — **Method-Driven**

  Objective(s):

  Humans may be exposed to compounds in the diet or in the environment that disrupt endogenous endocrine responses in various tissues. We propose to utilize cell biological approaches to determine the role of human cytochromes P-450, UDP-glucuronosyltransferases, and sulfotransferases in the antiestrogens. The relative abilities of the various human enzyme systems expressed by individual cell lines to alter the extent of green fluorescent protein synthesis will indicate those human enzyme activities that activate or deactivate endocrine disrupting agents.

- **Photoinduction of Cutaneous Malignant Melanoma in TP-ras/ink4A (+/-) Transgenic Mice** — **E0708901** — **Predictive Toxicology**

  Objective(s):

  1) Characterize photochemical DNA damage in the skin of TP-ras/ink-4a mice exposed to UVA+UVB radiation;
  2) Determine whether cutaneous malignant melanoma can be induced in neonatal TP-ras (+) ink4a (+/-) transgenic mice using UVA+UVB radiation;
  3) Identify photochemically induced mutations within the ink4a/p16/CDKN2A and p53 loci in tumor tissues; and,
  4) Determine whether UVA+UVB exposure at an early age creates a greater risk for developing cutaneous melanoma in TP-ras (+)ink4a(+/-) mice compared with chronic UVA+UVB exposure of older animals.

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Concept Papers

Title	Project Number	Strategic Research Goal

PI: Delclos, Kenneth

- **Concept - Protective Effects of Soy-containing Diets Against Renal Toxicity: Implications for Animal Toxicity Assessments** — **E0714201** — **Agent-Driven**

  Objective(s):

  Examine the effects of diet and timing of exposure on renal toxicity induced by nonylphenol, di(2-ethylhexyl)phthalate (DEHP), and mono(2-ethylhexyl)phalate (MEHP).

- **Optimization of Procedures for 1) laser capture microdissection of rat kidney for gene and protein expression studies and 2) measurement of renal cyclooxygenases, antioxidant enzymes and isoprostanes** — **P00619** — **Method-Driven**

  Objective(s):

  1) Determine optimal parameters for laser capture microdissection to collect distinct renal cell populations for analysis of mRNA and proteins;
  2) Optimize conditions for the measurement of cox-1, cos-2, glutathione peroxidase, superoxide dismutase and quinone reductase; and,
  3) Evaluate the feasibility of utilizing commercial ELISA kits for the determinations of prostaglandin and isoprostane levels in renal cortex and medulla.

PI: Roberts, Dean

- **Concept - Two-Dimensional micro-LC Proteomics using Stable-isotope Affinity Tags for Differential Display of Toxicity-induced Biomarkers** — **E0710301** — **Method-Driven**

  Objective(s):

  Investigate changes in protein expression profiles using two-dimensional LC, stable-isotope coded affinity tags, and mass spectrometry for differential display of biomarkers associated with doxorubicin-induced mitochondrial toxicity.

Biometry and Risk Assessment

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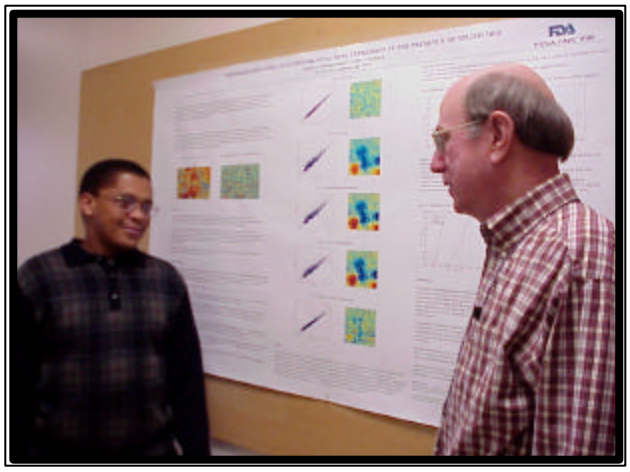

Drs. Molefe and Kodell discuss statistical methods for cDNA microarray data.

Executive Summary

Introduction

The Division of Biometry and Risk Assessment conducts research to address FDA's regulatory needs for new and improved methods for the assessment of human health risks resulting from exposure to chemicals, microorganisms and radiation. The Division is comprised of mathematical statisticians, research biologists, computational chemists and information technology specialists. In addition to conducting individual and collaborative research within the Division and across NCTR, Division scientists collaborate with scientists at other FDA centers, other government agencies and academic institutions.

FY 2002 Accomplishments

During FY 2002, scientists in the Division engaged in research addressing a variety of risk-assessment issues relevant to science-based regulation. Research projects included the following:

- analyzing and interpreting gene expression data from cDNA microarray experiments
- developing computer-based systems to predict the toxicity of untested chemicals
- assessing the risk of skin cancer due to interactions of cosmetics with sunlight
- modeling the spread of infection and disease caused by foodborne pathogens
- classifying individuals according to enzymatic biomarkers of disease risk
- enhancing pharmacokinetic simulation software to simultaneously model a parent chemical and several metabolites
- developing quantitative methods for assessing the cumulative risk from exposure to mixtures of chemicals
- developing improved statistical tests for assessing tumorigenicity in long-term bioassays.

Of special note in FY2002 was the establishment of the Center for Toxicoinformatics within the Division of Biometry and Risk Assessment. This center was established to ensure proper integration and coordination of informatics needs and capabilities at NCTR related to the massive amount of data arising from new technologies in genomics, proteomics and metabonomics.

Project Number Codes:
E–Ongoing P–Preliminary S–Support

FY 2003 Plans

For FY 2003, strong emphasis will continue to be placed on research stimulated by both the explosion of information in the post-genomic era and by the continuing threat of bioterrorism. Scientists in the Division will conduct research related to the analysis and interpretation of cDNA gene expression microarrays, research that addresses issues in toxicoinformatics and data mining, and research on the spread and detection of microbiological agents of terrorism.

Research projects related to microarrays include:

- developing network architecture to identify precursor genes, co-expressed genes and target genes for constructing genetic profiles;
- developing statistical adjustments for the testing of multiple genes for differential expression among comparison groups; and,
- performing microarray analysis of formalin-fixed tissues from dietary restriction studies.

Research projects related to toxicoinformatics and data mining include:

- developing a computer-based system that integrates databases, libraries and analytical tools for managing and analyzing "omics" data; and,
- developing computer-based systems to predict organ-specific toxicity using multiple inputs based on chemical structures and spectra.

Research projects related to microbiological agents of terrorism include:

- developing statistical methods to identify microbial pathogens based on protein spectra; and
- developing models to predict the spread of microbial pathogens through a population.

In addition to the above highlighted projects, research will continue on all other active projects. Center-wide consultation on statistical, pharmacokinetic and toxicoinformatic problems will continue, as will the provision of oversight to on-site contract activities associated with statistical analyses and experimental support.

Public Health Significance

The Division of Biometry and Risk Assessment is a focal point within the FDA for research in the area of health risk assessment. Human health risk estimates impact the regulation of exposure to toxic substances, thereby affecting both the health of the U.S. population and the health of the U.S. economy. The nature of the research carried out in the Division is diverse, with projects characterized by development of mathematical and statistical theory and methods for risk assessment; biological experimentation and pharmacokinetic modeling with specific agents; and development of computational systems for predicting toxicity through knowledge discovery in databases. The ultimate goal of the research carried out in the Division is to improve the regulation of natural or synthetic toxic substances occurring in foods, drugs, cosmetics, biologics, medical devices and animal drugs. Continued significance to the FDA is fostered through interactions with individuals and committees at other FDA centers that are involved in evaluations of risk for the regulation of specific products. Participation by Division scientists on interagency risk-assessment committees and panels ensures relevance of the division's research to broad public health issues.

Research Projects

Title	Project Number	Strategic Research Goal
PI: Chen, James		
◆ **Cumulative Risk Assessment for Chemical Mixtures**	**E0708701**	**Predictive Toxicology**
Objective(s): Develop and apply the relative potency factors approach for estimating the risk from combined exposures to a set of chemicals having a common mode of action.		
◆ **Experimental Design and Analysis of GeneArray Expression Data**	**E0711201**	**Method-Driven**
Objective(s): Develop statistical and computational procedures for the design, analysis, and interpretation of gene expression data from microarray experiments.		
PI: Delongchamp, Robert		
◆ **A Mixture Model Approach to Classifying CYP1A2 Variants that Adjusts for their Current Smoking Status**	**E0703701**	**Method-Driven**
Objective(s): 1) Examine statistical methods for parametric density estimation based upon a mixture of normal distributions; and, 2) Apply the method to a data set where hepatic cytochrome P4501A2 activity appears to be induced by smoking cigarettes.		
◆ **An Investigation of the Effects of Adjusting Intensities from cDNA Arrays on the Assessment of Differential Gene Expressions**	**E0709601**	**Method-Driven**
Objective(s): 1) Evaluate the advantages/disadvantages of using either the mean or median for normalizing array data in the presence of nuisances; 2) Determine an optimal size of subsets for normalizing data in the presence of nuisances that merit their use; and, 3) Assess the bias induced by nuisances and the extent to which normalization procedures are able to remove them.		

Title	Project Number	Strategic Research Goal

PI: Kodell, Ralph

- **Attribution of Tumor Lethality in the Absence of Cause-of-Death Information** — **E0689601** — **Method-Driven**

  Objective(s):

  1) Develop a nonparametric procedure for estimating distributions of time to onset of and time to death from occult tumors in the absence of cause-of-death information;
  2) Develop a method for entering the number of fatal tumors in an experiment that lacks cause-of-death data, in order to modify the IARC cause-of-death test;
  3) Develop a procedure for estimating the lag time between onset of and death from an occult tumor, when cause-of-death data are unavailable; and,
  4) Illustrate the new procedures using data from the PCR studies.

- **Dose-Response Modeling for Microbial Risk Assessment** — **E0704501** — **Predictive Toxicology**

  Objective(s):

  1) Evaluate existing dose-response models for microbial risk assessment;
  2) Develop improved models for estimating probabilities of infection and disease; and
  3) Develop methods for incorporating model uncertainty into microbial risk assessment.

- **Statistical Analysis of Tumor Multiplicity Data** — **E0706101** — **Predictive Toxicology**

  Objective(s):

  1) Investigate the model of Kokoska, *et al.* for analyzing tumor multiplicity data from single-induction experiments, using the negative binomial distribution for the number of induced tumors and the Weibull distribution for the time to observation of such tumors;
  2) Develop a likelihood-ratio approach, adapted from the model of Kokoska, et. al. For testing between-group differences with respect to the expected number of induced tumors as well as the distribution of time to observation;
  3) Develop tests for dose-related trend with respect to the expected number of induced tumors and the distribution of time to observation;
  4) Extend the model to situations involving multiple or continuous dosing, and situations in which there is a background of spontaneous tumors;
  5) Conduct a Monte Carlo simulation study to compare the new methodology to conventional analytical approaches, and to evaluate its robustness, and identifiability; and,
  6) Develop user-friendly software for easy implementation of the proposed analytical procedures.

Title	Project Number	Strategic Research Goal

- **Interagency Agreement on Developing and Evaluating Risk Assessment Models for Key Waterborne and Foodborne Pathogens and Chemicals** — **P00422** — **Predictive Toxicology**

  Objective(s):

  Develop and evaluate risk assessment models and chemical risk assessments for food and water. This is a proposal for a new interagency agreement between NCTR and EPA's National Center for Environmental Assessment.

PI: Tong, Weida

- **General Support for Center for Toxicoinformatics** — **S00617** — **Center Support**

  Objective(s):

  Provide toxicoinformatics support for the center-wide research.

PI: Turturro, Angelo

- **Development of a Model for the Transmission Kinetics of Infection by Cryptosporidium parvum with Acquisition of Data on Key Parameters** — **E0708201** — **Concept-Driven**

  Objective(s):

  1) Standardize the virulence of doses of Cryptosporidium parvum used in this and subsequent studies;
  2) Investigate the suitability of the Brown-Norway rat as a model for Cryptosporidium parvum infectivity in humans, or the C57Bl/6 mouse chemically suppressed with dexamethasone if BN is unsuitable;
  3) Compare Cryptosporidium parvum infectivity for model animals with age and pregnancy, which may influence immunocompetence;
  4) Compare Cryptosporidium parvum infectivity for model animals with treatment with chemicals which induce immunosuppression other than by dexamethasone;
  5) Compare Cryptosporidium parvum infectivity in animals with immunosupression models similar to the effects of AIDS;
  6) Compare Cryptosporidium parvum infectivity in animals with physiolgical stress and nutritional immunosupression models; and,
  7) Use these data in pathogen virulence and host susceptibility in a model for the transmission dynamics of Cryptosporidium parvum in human outbreaks.

PI: Young, John

- **Computational Predictive System for Rodent Organ-Specific Carcinogenicity** — **E0708301** — **Predictive Toxicology**

  Objective(s):

  Using modern SAR technology and statistical approaches, an expert system can be developed to predict rodent carcinogenicity.

Title	Project Number	Strategic Research Goal

- **Bio-Preg to Windows 2000 Upgrade** **E0713001** **Method-Driven**

  Objective(s):

  Upgrade Bio-Preg to a Windows-based program which will be called Win-Preg.

- **Species Comparison Utilizing a PBPK Model** **P00393** **Predictive Toxicology**

  Objective(s):

  Pharmacokinetic data from the literature will be excerpted and adapted to be simulated via a PBPK model. Initially the literature data will be limited to dexamethasone, cocaine, and methylmercury. Species comparisons will be made utilizing this single pharmacokinetic model.

Publications

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Chemistry

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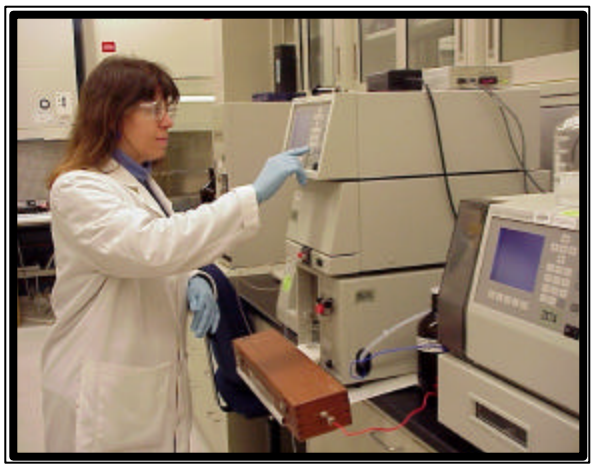

Chemist Beth Brown performs a dose certification HPLC analysis in support of an FDA-nominated NTP study.

Executive Summary

Introduction

The Division of Chemistry contributes to NCTR/FDA-directed research initiatives through investigations on sensor technology for rapid screening to assess food quality and detect volatile explosives, nuclear magnetic resonance (NMR)-based metabonomics, mass spectrometry (MS) applications in proteomics, biomarkers, analytical chemistry and counter-terrorism, predictive toxicology using computational chemistry and artificial intelligence, as well as analytical chemistry collaborations for the National Toxicology Programs (NTP). The Division has made investments in state-of-the-art equipment in MS and a high-field NMR instrument for the establishment of proteomics and metabonomics facilities. These facilities and new techniques will complement the newly created program centers in structural and functional genomics, toxicogenomics, the Heptatoxicity Lab, and other research divisions at NCTR to elucidate mechanisms of action of chemicals and products under question.

FY 2002 Accomplishments

The newly created Mass Spectrometry Proteomics Laboratory has rapidly transformed from its conception in 2001 into an established laboratory in 2002. Two scientists with substantial experience in proteomics have been hired with a third position to be filled soon. The laboratory houses state-of-the-art MS equipment, a matrix-assisted laser desorption ionization–time-of-flight (MALDI-TOF), an electrospray ionization–quadropole time-of-flight (ESI-Q-TOF), and an ion trap with both MALDI and ESI capabilities, as well as a full line of robotics to aid in sample processing. Both ESI instruments are equipped with custom-designed nanoHPLC sources that allow high sensitivity required for most proteomic experiments.

The Mass Spectrometry Counter Terrorism Program was initiated in 2002 with the purchase and installation of a MALDI-TOF and a pyrolysis metastable atom bombardment (MAB)-TOF MS. These instruments will be utilized to produce unique mass spectra of microorganisms, and in conjunction with pattern recognition, rapidly characterize bio-terror agents or bio-terror hoax materials such as talc or flour.

Project Number Codes:
E–Ongoing P–Preliminary S–Support

In computational chemistry, pattern recognition-based methods for the non-invasive diagnosis of brain tumors were developed in collaboration with researchers at the University of Arkansas for Medical Sciences (UAMS). A patent was submitted for three-dimensional quantitative spectrometric data-activity relationship (3D-QSDAR) (E07126), where models are developed using the combination of NMR spectral information with internal structural connectivity information and relating this combined pattern of information to biological endpoints. Advanced Chemistry Development (ACD) in Toronto, Canada, has negotiated a license for the previous SDAR patent (E07068) and is expected to license the patent for 3D-QSDAR.

Pharmacokinetic studies were done with the University of Pittsburgh and the UAMS on hyperforin (HF), a major active component of St. John's wort, which may affect drug-herbal interactions that diminish therapeutic efficacy of medications (E0705611). The methods developed to measure HF in plasma will aid to assess the effects of St John's wort on human xenobiotic enzymes.

In a caloric restriction study, a 10-percent reduction in calories significantly increased the life span of experimental animals (E692401), possibly through reduced free radical damage that may contribute to aging and disease since antioxidant potential increased. Moreover, antioxidant enzyme potential and mitochondrial function increased in obese women who have undergone gastric bypass surgery (E699101), suggesting that reduction in caloric intake (even small reductions) may be beneficial in humans.

Rapid, chemical sensor technology (designated as Food Quality Indicator [FQI] to assess food quality (E0708001) was evaluated by the National Marine Fisheries and Canadian Center for Fisheries Innovation (CCFI), St. John, Newfoundland, Canada. The commercial version of Fresh Tag™ was more rapid, sensitive and rugged than other comparative methods tested by CCFI. An interagency agreement was established with the Federal Aviation Administration (FAA) to develop similar rapid sensor methods to detect explosives for protection of the air transportation industry (E0708101).

Solid phase and immunoaffinity procedures, followed by liquid chromatography electrospray ionization (LC-ESI) tandem MS methods, were developed to isolate and quantitate the dietary and tobacco carcinogens heterocyclic aromatic amines (HAAs) and their metabolites in human urine of population studies that are at elevated risks of cancer development.

The Analytical Support and Mass Spectrometry Branches continued collaborations on chemicals under investigation by the NTP, including the *Aloe barbadensis* constituents, aloin A and malic acid, and several anti-HIV drugs. The Division collaborated with the Center for Veterinary Medicine (CVM) on analysis of the antibiotic erythromycin A (EA) in salmon (E06980) and provided guidance to CVM for inter-laboratory method trials for leucomalachite green and multi-sulfonamide methods. The Mass Spectrometry Branch also continued collaborations on NTP initiatives on isoflavones (E02138), fumonisins and ethinylestradiol in dose feed (E02138), and methods development of anti-HIV drugs (E02141). The branch also conducted characterization of HF degradation products (E07056) and validation of a LC-electrochemical method for EA in salmon (E06980). In collaboration with the Division of Microbiology, microbial or fungal metabolites of EA (E07075), daidzein (E07007), fluoroquinolones (E07052) and polycyclic

aromatic hydrocarbons (E07075) were characterized by MS to understand mechanisms of antibiotic and antimicrobial resistance and biodegradation of environmental contaminants.

FY 2003 Plans

Several new research initiatives are planned for 2003. In Proteomics:

- Identify protein markers associated with the toxicity of acetaminophen and other hepatotoxins (with the Hepatoxicity Lab);
- Identification and quantification of proteins (enzymes) involved in antibiotic resistance and in biodegradation of environmental contaminants (with the Division of Microbiology); and,
- Investigations on mechanisms of protein changes and modifications as they relate to aging, free radical damage and disease states, caloric restriction and obesity (intra- and inter-divisional collaborations).

In Counter-Terrorism:

- Develop of MS methods, coupled with pattern recognition approaches to rapidly detect microorganisms as bioterror agents or hoax materials and cross-validate with microbiological and genetic methods (with the Division of Microbiology).

In Computational Chemistry:

- Develop computational methods to diagnose breast tumors using magnetic resonance (MR) and ^{13}C MR scans. These scans are biochemical spectral tissue profiles, which can be thought of as spectral "fingerprints" and can be used by pattern recognition methods to diagnose disease (E07136).

In NMR-Based Metabonomics:

- A research program on metabonomics has been initiated to assess chemicals/drugs that may influence metabolism and homeostasis. Acetaminophen, a well-known hepatotoxin, will be the first drug investigated (E07150) in collaboration with Dr. Y. Dragan, and PIs in Functional Genomics, Proteomics, Biometry, CDER, and investigators at the UAMS.

Public Health Significance

The new technologies in MS applications for proteomics, biomarker development and counter-terrorism, NMR-based metabonomics, and computational chemistry that are under development in the Division of Chemistry will aid in addressing many health and food safety issues. MS methods in proteomics will advance studies on mechanisms of antibiotic resistance, biochemical modifications of proteins associated with cellular metabolism and protein function, and signature proteins as biomakers of disease states that are under study at NCTR. MS is also a critical means to assess exposure to environmental toxins and an emerging technology to rapidly identify bioterror-related materials. NMR-based metabonomics is a non-invasive method that can measure the kinetic profile of onset, progression, and recovery from toxic events induced by drugs. The

data obtained can aid in the interpretation of biological effects of drugs and in the establishment of FDA policies during an investigational new drug (IND) or new drug applications (NDA) submission to the FDA. The use of computational chemistry methods in examining patterns of diseases, such as breast cancer, may enable us to identify disease states at a much earlier juncture, perhaps before the onset of pathological abnormalities, and lead to earlier treatments. All of these programs are using exciting, state-of-the-art technologies to improve human health and safety.

Research Projects

Title	Project Number	Strategic Research Goal

PI: Ang, Catharina

- **ADDEND: Development of Analytical Methodologies for Assessing Bioactive Herbal Ingredients in Functional Food Systems** — **E0705611** — **Method-Driven**

  Objective(s):

  Expand the original protocol to include functional foods as additional substrates and to include active components of Echinacea and marker compounds as analytes. The scope of this protocol addendum covers the analytical methodology development aspect for St. John's Wort and Echinacea. Functional food items to be investigated may include tea, drink, soup, snack, cereal and candies.

- **Influence of Hyperforin Concentration on Drug Interactions** — **P00436** — **Method-Driven**

  Objective(s):

  Quantify hyperforin concentrations in plasma samples collected at the UAMS as a part of studies evaluating drug interactions between St. John's Wort and the conventional medicines.

PI: Beger, Richard

- **Producing Spectrometric Data Activity Relationship (SDAR) Models for Compounds Binding to Receptors of Toxic Responses.** — **E0706801** — **Predictive Toxicology**

  Objective(s):

  To produce Spectometric Data-Activity Relationship (SDAR) models that use only experiment 13C NMR and EI/MS data to predict whether a compound has a specific binding affinity to a specific receptor or toxicological responses. The major benefit of the experimental SDAR approach is its flexibility since the spectral data can be used for other toxicological systems.

Title	Project Number	Strategic Research Goal

- **Methods for Predicting Toxicological Properties of Molecules from Their NMR Chemical Shifts Through-bond and Through-space Distance Connectivity Patterns** — **E0712601** — **Predictive Toxicology**

  Objective(s):

  Produce models that use NMR data and infuses three-dimensional atom-to-atom through-bond connectivity and atom-to-atom through-space intramolecular distance information into a three-dimensional pattern that can be used by pattern recognition software to build a model of a biological or toxicological endpoint. The results of the 3D-QSDAR models will be compared to the results of QSDAR and QSAR models from protocols E0706801, E707701 and E0708301.

PI: Billedeau, Stanley

- **Development of Methods for Analysis and Confirmation of Erythromycin A Residues in Tissue Samples from Terrestrial and Aquatic Farmed Animals by Liquid Chromatography** — **E0698001** — **Method-Driven**

  Objective(s):

  The principal objective of this project is to develop determinative and confirmatory analytical chemical procedures, using high performance liquid chromatography/electrochemical detection and high performance liquid chromatography/atmospheric pressure chemical ionization mass spectrometric detection, for Erythromycin A in biological samples taken from agricultural animals. Specifically, the goal is to develop complete methods for the analysis of Erythromycin A in muscle and liver tissues from poultry, non-processed bovine milk, and muscle tissues from salmon, catfish and shrimp. Sensitivity levels for these methods are expected to be at least 100 parts per billion for liver tissue and 50 parts per billion for muscle tissue and milk as requested by the Center for Veterinary Medicine.

PI: Buzatu, Dan

- **Comparison of Principal Components Analysis (PCA) and Artificial Neural Networks (ANN) for Prediction of Qualitative and Quantitative Biological End Points from Spectrometric Data** — **E0707701** — **Predictive Toxicology**

  Objective(s):

  This study will introduce and evaluate a new ANN-based method for the correlation of spectrometric data to biological endpoints/activities. The evidence and methodology needed to expand the existing FDA-owned patent covering the use of spectrometric data for predicting biological endpoints will be provided.

Title	Project Number	Strategic Research Goal

PI: Feuers, Ritchie

- **Influence of Dietary Restriction on Somatic Mutation and Antioxidant Enzymes Induced by Exposure of Female and Male Fischer 344 Rats to Bleomycin (BLM)** — **E0699101** — **Predictive Toxicology**

  Objective(s):

  1) Determine the frequency of occurrence of lymphocytes bearing a mutant form of the hprt gene as an indicator of DNA damage in caloric restricted and in *ad libitum* rats following exposure to bleomycin (BLM);
  2) Determine how the activity of antioxidant enzymes such as catalase, glutathione peroxidase, and glutathione reductase relates to the mutant frequencies determined from the above objective;
  3) Determine the activity of the electron transport systems as an indicator of mitochondrial function during drug exposure; and,
  4) Evaluate the integrity of mitochondrial DNA in BLM-treated rodents.

- **Memphis Study: Evaluation of Calorically Restricted Human Surgical Samples Received from Department of Surgery University of Tennessee, Memphis** — **E0699801** — **Predictive Toxicology**

  Objective(s):

  Determine whether rodents and humans behave biologically in the same manner when calorically deprived but nutritionally supplemented.

PI: Leakey, Julian

- **Chronic Bioassay of Chloral Hydrate in Male B6C3F1 Mice Using Idealized Body Weight Curves that are Normalized by Modulation of Caloric Intake** — **E0211701** — **Concept-Driven**

  Objective(s):

  1) Determine the chronic toxicity and potential carcinogenicity of chloral hydrate administered by aqueous gavage, to male $B6C3F_1$ mice; and,
  2) Determine the feasibility of utilizing dietary control (i.e., the manipulation of caloric intake) to control body weight gain so that all mice in each experimental group of the bioassay conform to an idealized weight curve.

- **ADDEND: Dose Response to Chloral Hydrate in Dietary Restricted Mice** — **E0211722** — **Concept-Driven**

  Objective(s):

  Determine the effect of two levels of dietary restriction on the pharmacokinetics, metabolism and acute hepatotoxicity of chloral hydrate in male B6C3F1 mice.

Title	Project Number	Strategic Research Goal

PI: Miller, Dwight

- **Development of Devices/Methods for Determination of Food/Seafood Quality** **E0687401** **Method-Driven**

  Objective(s):

  Assist FDA with problems incurred in testing seafood for decomposition by developing an expeditious assay for determining volatile and semivolatile organic compounds in spoiled seafood.

- **Innovative Methods for Determining Food Quality: Decomposition, Safety and/or Economic Fraud** **E0699701** **Method-Driven**

  Objective(s):

  1) Examination of the total volatile bases (TVB) and putrescine (PU), cadaverine (CD) and histamine (HS) methods for potential regulatory use and validation of TVB as an indicator of decomposition; and,
  2) Develop rapid detection methods for the determination of decomposition analytes in seafood.

- **Rapid Screening Test for Food Quality** **E0708001** **Predictive Toxicology**

  Objective(s):

  Develop simple, field-compatible methods to test for food quality.

- **Application of Solid Phase Detection Systems to Explosives in Airplane Cargo** **E0708101** **Method-Driven**

  Objective(s):

  1) Detection of ammonia - formulation, measurement of Am concentrations around container of ammonium nitrate, reformulation of Fresh Tag chemistry for label-type detection, and development of PE or PVC film Shrink Rap detector;
  2) Detection of acids, and,
  3) Detection of oxidizers such as peroxides and NO or NO_2.

Title	Project Number	Strategic Research Goal

PI: Turesky, Robert

- **Human Risk Assessment of Heterocyclic Aromatic Amines: Exposure, Development of Novel Biomarkers of Cytochrome P450 1A2 Activity and DNA Adduct Formation** — **E0709101** — **Agent-Driven**

Objective(s):

1) Analyze HAAs by HPLC-MS in previously unreported grilled foods that are indigenous to southern cooking style, including Cajun-type foods;
2) Establish sensitive biomarkers for interspecies extrapolation and human health risk by utilizing HPLC-MS methods to measure metabolites and excised DNA adduct of MeIQx and PhIP in human urine for cohort studies;
3) Determine if specific metabolites of MeIQx and PhIP in human urine are catalyzed by P450 1A2, which is believed to be the major P450 involved in the toxication of these chemicals;
4) Evaluate the effect of chemoprotective agents and dietary supplements on enzyme modulation, and its impact on HAA metabolism and DNA adduct formation in human hepatocytes for eventual chemoprotective studies *in vivo*; and,
5) Use interspecies metabolism to assess the capacity of human and rat P450 1A2 orthologues in metabolic activation and detoxication of HAAs to assess human risk.

- **Toxicological Effects of Ochratoxin A** — **E0709401** — **Predictive Toxicology**

Objective(s):

1) Establish chemical and biological markers of oxidative stress to proteins using biochemical and mass spectrometry techniques;
2) Establish markers of oxidative damage to DNA by measurement of abasic site formation and oxidized DNA lesions by affinity detection and LC-MS methods;
3) Investigate changes in gene expression and protein expression in liver and kidney as a function of OTA treatment; and,
4) Correlate differences in these above endpoints with *in vivo* mutagenesis using the Big Blue Rat experimental model.

Title	Project Number	Strategic Research Goal

PI: Wilkes, Jon

- **First Phase Development of a Rapid Screening Method for Identification of Complex Mixtures by Pyrolysis-Mass Spectrometry with Computerized Pattern Recognition** — **E0693101** — **Knowledge Base**

  Objective(s):

  Evaluate feasibility of the application of pyrolysis mass spectrometry (PyMS) with computerized pattern recognition (PattRec) for the rapid identification of a sample (a) which is a complex chemical mixture, (b) which is member of a set of such mixtures, and (c) for which there is a regulatory need to distinguish the individual members of the set. Typical examples of applications: (a) the rapid identification of culturable pathogenic and non-pathogenic bacteria in food, (b) the distinction of adulterated from pure foods or cosmetics, or of generic from brand name pharmaceutical products, or (c) demonstrating the virginity of plastic materials used in food containers.

- **Combining MAB/MS with Pattern Recognition to Sub-type Bacteria** — **E0707901** — **Method-Driven**

  Objective(s):

  This work is intended to demonstrate the validity of the combination of pyrolysis/metastable atom bombardment (MAB)/mass spectrometry (PyMAB/MS) with computerized pattern recognition (PattRec) for bacterial sub-typing. The work should produce a scientifically and technologically validated basis for commercial licensing of an NCTR-patented process: a method for assembling coherent spectral data bases for use in rapid chemotaxonomy at the strain and sub-strain level.

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Project Number Codes:
E–Ongoing P–Preliminary S–Support

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Concept Papers

Title	Project Number	Strategic Research Goal
PI: Beger, Richard		
◆ **Concept - NMR-Based Metabonomics of Drugs Suspected of Liver and Kidney Toxicity**	**E0715001**	**Predictive Toxicology**
Objective(s): Produce NMR-based metabonomics studies of liver and kidney toxicity.		
PI: Buzatu, Dan		
◆ **Concept - 13C Magnetic Resonance Breast Cancer Diagnostic Models**	**E0713601**	**Predictive Toxicology**
Objective(s): Develop the high-field magnet 13C Magnetic Resonance pulse sequences necessary to obtain single voxel MR scans of rat breast tumor tissue. Data from the tumor MR scans and in combination with gene array data will be used in conjunction with advanced pattern recognition software to develop breast cancer diagnostic models.		
◆ **The Development of Dynamic Mass Spectral/Pattern Recognition Based Methods for the Rapid Identification of Bioterror Agents**	**E0714601**	**Method-Driven**
Objective(s): Develop the necessary computational capability to enable the rapid identification of pathogen/non-pathogen microorganisms, non-biological hoax materials, and mixtures of all mentioned collected in real-world situations. An analysis will be done of the salient spectral features necessary for identifying these substances, and the effect of both instrumental and pattern definition techniques on the ability to use these features for rapid identification.		
PI: Feuers, Ritchie		
◆ **Concept - Development of Techniques in Proteomics for Use in Chemistry Studies**	**E0713501**	**Predictive Toxicology**
Objective(s): Develop techniques in proteomics with the goal of utilizing such techniques in genetic toxicology studies.		

Title	Project Number	Strategic Research Goal

PI: Turesky, Robert

- **Dietary Factors in the Etiology of Human Cancer, Agent-Driven Biomonitoring of Heterocyclic Aromatic Amines – CRADA-funded portion of E0709101** — **E0709102**

  Objective(s):

  1) Determine the extent of heterocyclic aromatic amine (HAA) exposure via urine analysis and determine whether HAA may contribute to human cancer development based upon the nested case-control studies;
  2) Develop analytical methods to measure the HAA metabolites and DNA adducts in urine; and,
  3) Correlate HAA metabolite profiles with genotype and phenotype data associated with xenobiotic enzymes associated with cancer risk, such as cytochrome P450 1A2, N-acetyltransferases.

- **Rapid Analysis of Protein Oxidation Markers by ImmunoDetection and Mass Spectrometry** — **P00614** — **Method-Driven**

  Objective(s):

  1) Develop PAGE and 2-D gel methods to measure oxidative damage to proteins *in vitro* and in experimental animal models (rodents);
  2) Establish selective methods to measure protein oxidative damage by PAGE and 2-D gels using immunodetection techniques and determine limits of detection;
  3) Establish rapid immunoaffinity chromatography methods to isolate oxidized proteins and peptides from animal tissues to assess oxidative status;
  4) Establish novel, solid-phase extraction approaches, instead of costly immunoaffinity chromatography techniques, to isolate oxidized proteins and peptides from animal tissues to assess oxidative status; and,
  5) Determine if rapid extraction techniques can be used to identify specific proteins/peptides by mass spectrometry approaches.

Title	Project Number	Strategic Research Goal

PI: Wilkes, Jon

- **Evaluation of Pyrolysis MAB/Tof MS and MALDI/Tof MS for Rapid Characterization of Presumptive Bio-terror Agent Samples** — **E0714701** — **Method-Driven**

Objective(s):

The suitability of mass spectral data obtained from both pyrolysis metastable atom bombardment MS and matrix-assisted laser desorption/ionization time-of-flight MS techniques will be evaluated for the purpose of rapidly characterizing presumptive bio-terror agent samples. This includes analysis of the salient spectral features necessary for identifying microorganisms from contaminated samples and differentiating tainted samples from hoax sample materials collected from the environment, as well as evaluating the effects of both instrumental and pattern-definition techniques on the ability to use these features for rapid identification.

Genetic and Reproductive Toxicology

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The genomics team from the Functional Genomics Center in the Division of Genetic and Reproductive Toxicology.

Executive Summary

Introduction

The Division of Genetic and Reproductive Toxicology (DGRT) conducts applied basic research to address specific high-priority issues regarding genetic or reproductive/developmental toxicology. Division research is directed toward developing and validating new methods that can be used for the identification of potentially hazardous food additives, human and animal drugs, biological therapies and medical devices. In addition, in collaboration with other NCTR scientists, DGRT utilizes the methodologies that it develops to conduct research to understand the potential toxicity of specific high-priority drugs, dietary supplements and/or other agents. Genistein, tamoxifen and alpha-hydroxytamoxifen, the AIDS therapeutic drugs zidovudine and lamivudine and the fungicide malachite green are currently undergoing extensive evaluations in cross-division collaborative research efforts.

Currently there are four basic focus areas in the Division research program. Genetic Toxicology research addresses the development of methods to assess the potential for chemicals to negatively impact human genetic material or the function of the genetic material. Reproductive/Developmental Toxicology focuses on methods to understand normal human development and how chemicals might alter normal development. In addition to these disciplinary research areas, the Division conducts research to understand the impact of dietary supplementation. This research primarily focuses on understanding the physiological and genetic consequences of dietary modulation. In 2002 the Division initiated a new research focus to utilize new molecular approaches to evaluate the level of gene expression within entire cells or tissues.

FY 2002 Accomplishments

Methods developed by DGRT scientists were recognized by their inclusion in the new book, **Methods in Molecular Biology: Molecular Toxicology Protocols**, published by The Humana Press. These new methods include:

- an *in vivo* mouse model developed to evaluate chemical-induced mutations utilizing a gene in its natural location rather than as an artificially inserted gene (a transgene);
- a new molecular technique that can detect specific, rare mutational events in cancer genes (oncogenes); and

Project Number Codes:
E–Ongoing P–Preliminary S–Support

- a molecular technique that can be used in humans to detect rare, but specific, chromosomal re-arrangements associated with human blood cell cancers.

DGRT research was featured in two issues of the new on-line FDA/NCTR journal: **Regulatory Research Perspectives: Impact on Public Health**. The web address for this journal is: http://www.fda.gov/nctr/science/journals/Default.htm. The November 2001 issue featured a new technique that can be used to directly measure specific mutations in genes (oncogenes) directly involved in tumor induction. The induction of a mutation is generally the initiating event in the development of a tumor, and that specific mutation will be present in every cell of the tumor. However, tumor development requires multiple mutations. Using their newly developed method DGRT scientists showed that they can detect secondary mutations in mouse liver tumors and also that they can detect these important mutations prior to the development of a tumor. Such mutations can be detected when they occur in as few as 1 cell in 100,000 cells.

The May 2002 issue featured a collaborative project between NCTR and the U.S. Environmental Protection Agency (EPA). Both FDA and EPA have regulatory authority for chemicals in foods and the environment that interfere with normal hormone functions. In a collaborative project with the EPA Office of Pollution Prevention and Toxic Substances, NCTR scientists have developed a quantitative structure-activity relationship (QSAR) approach that can be used to screen thousands of chemicals and determine the likelihood that they would be estrogenic. A series of QSAR models were developed and validated against experimental data. This QSAR approach was then applied to three environmental data sets identified by EPA, and to a list of chemicals of concern supplied by CFSAN and CDER. This QSAR screen provided a list of priority chemicals for further experimental evaluation or regulatory decision making.

Division scientists contributed to several studies involving specific chemical exposures. Phytoestrogens are receiving much attention because of their potential ability to supplement the natural but declining levels of estrogen in menopausal women. Based on cell culture studies, DGRT scientists have shown that genistein (a component of soy products) can cause mutation. This observation was further investigated using whole animal studies. In one study there was no evidence that genistein can cause mutations to specific reporter genes. In the other study, no chromosomal damage was observed in the tissues analyzed, but there was some alteration in cell growth. Taken together, these studies indicate that while there was no detectable damage to the genetic material, genistein or its metabolites did reach the tissues and thus may have the ability to induce more subtle types of cellular damage.

The antiestrogen tamoxifen is widely used for the treatment and prevention of breast cancer. However, there is evidence that the drug causes liver cancer via a mechanism involving the metabolite, alpha-hydroxytamoxifen, which is metabolized and reacts with DNA to produce mutations. In collaboration with other NCTR scientists, DGRT investigators have compared the types of mutations produced by tamoxifen and alpha-hydroxytamoxifen in the sensitive Big Blue® rat mutation assay and found that tamoxifen and alpha-hydroxytamoxifen produced similar mutations, which were different from those found in untreated animals. These results support the hypothesis that alpha-hydroxytamoxifen is the major metabolite causing the initiation of liver tumors in rats.

During 2002, the NCTR Functional Genomics Center was established. While laboratory renovations and equipment acquisitions were being completed, DGRT scientists collaborated with EPA scientists and successfully used microarray gene expression analysis to identify a number of genes altered in rodents given dichloroacetic acid, a known rodent carcinogen, in their drinking water. They identified specific genes that are involved in cell growth, tissue remodeling, apoptosis (normal cell death), cancer progression, and foreign chemical metabolism. This study demonstrates the potential utility of the new DNA microarray technology in evaluating the mechanisms by which chemicals exert their toxicity.

FY 2003 Plans

DGRT scientists will initiate the development of a new transgenic assay. They have developed a new cell line into which they inserted the gene for a fluorescent protein and another gene that controls its production. When a chemical causes a mutation in the controlling gene, the cell produces a fluorescent protein and becomes visible under UV light and can be quantified using cell-sorting instrumentation. Division scientists will investigate whether they can insert this gene into mice and develop a successful whole animal test system.

DGRT scientists will apply their new technology measuring rare specific mutations in cancer causing genes to a colon cancer and a skin cancer model.

The Functional Genomics Center will continue its validation of its microarray technology. This validation will include a collaborative project with the National Institute of Standards Technology (NIST). Projects utilizing microarray technology will also be initiated.

Work will continue on several ongoing projects including: (1) An Office of Women's Health project that is investigating whether genistein can decrease the induction of carcinogen-caused mutations; (2) collaborative project with the UAMS investigating the influence of biotin on the developing embryo; (3) project to investigate whether the drug azathioprine will increase the background level of mutations causing Lesch-Nyhan Syndrome and thus increase the incidence of this genetic disease in the offspring of individuals taking the drug; (4) NTP projects investigating the ability of the AIDS therapeutic drugs and malachite green to cause mutation in rodents; and (5) project to investigate whether the developing embryo and/or the neonate is particularly sensitive to the induction of mutation following exposure to known carcinogens.

Public Health Significance

Genetic toxicology is the investigation of the ability of chemicals to alter the genetic material. The FDA requires that petitioners provide data evaluating the potential genetic toxicity of their products as a part of the product approval process. Because genetic damage is believed to be important in tumor development, this information is used as a part of the evaluation of suspected carcinogens. Regulatory decisions are based not only on the identification of potentially genotoxic substances, but also on an understanding of their mode of action. Research within the Division centers on the development and validation of new methods by which to assess genetic risk. While tissue culture approaches are used to detect potential genotoxicity and to generate hypotheses concerning the basic mechanisms of genotoxicity, the Division specializes in the

development and validation of *in vivo* mammalian systems. An increased understanding of mutational mechanisms, combined with test systems with an increased ability to detect genetic damage, will provide the FDA with better information for decision-making. As new assays are validated, Division scientists work with international scientists to assure harmonization of protocols and the development of guidelines.

Reproductive/Developmental Toxicology is important to the Agency because one of the difficult challenges facing the FDA is the identification and regulation of chemicals, food additives, and biological therapies that may produce birth defects. Such defects affect 7% of the population at birth, another 7% have low birth weights, and at least 25% of pregnancies end in spontaneous abortion. The Division specializes in research to understand how toxicants may induce birth defects such as neural tube defects. Current research addresses the role that the vitamin folic acid may play in the normal closure of the neural tube. This research supports current thinking that diet may play a role in the development of normal offspring and that interactions between diet and toxicants may be important in producing certain birth defects.

In the future, the new genomic technologies will provide new tools for making better public health decisions. International research efforts are providing the scientific and medical community with an increased understanding of the genetic material and how it functions in both humans and rodents. Utilizing this information, new molecular technologies are being rapidly developed and can be used to evaluate structural and functional changes to the genetic material of both rodents and humans. The Division is developing the NCTR Functional Genomics Center. This capability will be available to NCTR and other investigators and will allow these technologies to be applied to fundamental risk assessment questions. While current technologies in the field of genetic and reproductive/developmental toxicology generally evaluate single endpoints, these new genomic technologies will provide the opportunity to detect alterations in a number of different endpoints. This new approach to evaluating toxicity will also allow for the integration of information across the various types of adverse health outcomes. For instance, when this technology is fully developed, it will be possible to concurrently evaluate chemicals for their ability to cause cancer, to impact the nervous system, to cause birth defects and to modify the immune function.

Research Projects

Title	Project Number	Strategic Research Goal

PI: Aidoo, Anane

- **Evaluation of the Effects of Daidzein and Genistein (Hormone Replacement Agents) on the Genotoxic and Carcinogenic Activity of the Model Mammary Carcinogen 7,12-dimethylbenz(a)anthracene (DMBA) in Ovariectomized Transgenic Big Blue Rats** — **E0707001** — **Predictive Toxicology**

  Objective(s):

  Determine whether daidzein and genistein or estradiol supplementation, singly or in combination to ovariectomized rats would alter in mammary tissues: a) DNA adducts produced by DMBA; b) the frequency and types of mutations produced by DMBA; and c) tumor formation by DMBA and types of p53 and H-ras mutations in tumors.

PI: Akerman, Gregory

- **Effect of p53 Genotype on Gene Expression Profiles in Mice Exposed to the Model Mutagen, N-ethyl-N'-nitrosourea (ENU)** — **E0712901** — **Concept-Driven**

  Objective(s):

  1) Determine the effect of mutation in the p53 tumor suppressor gene on gene expression profiles in young and aged mice; and,
  2) Determine the effect of mutation in p53 tumor suppressor gene on gene expression profiles in young and aged mice exposed to the model mutagen, N-ethyl-N-nitrosourea.

PI: Bishop, Michelle

- **Fluorescent-based Detection of Oxidative DNA Damage in Cells Treated *In vitro* Using Flow Cytometry and Fluorescence Microscopy** — **P00441** — **Method-Driven**

  Objective(s):

  1) Develop a sensitive and reliable method for the detection of 8OHdG in cells by flow cytometry and fluorescence microscopy;
  2) Optimize conditions for the assay; and,
  3) Apply the methods developed to evaluate free radical mechanism of drug-or-chemical-induced DNA damage in cells.

Title	Project Number	Strategic Research Goal

PI: Chen, Tao

- **Comparison of Mutation Induction and Types of Mutations in the cII Gene of Big Blue Mice Treated with Carcinogens as Neonates and Adults** — **E0709001** — **Predictive Toxicology**

  Objective(s):

  1) Determine the mutant frequencies in the cII gene of lambda/lacI transgenic mice treated with ethylnitrosourea, a direct-acting carcinogen, and the modifying role of age, sex and target organ;
  2) Compare the mutant frequencies in the cII gene of livers from the transgenic mice exposed as neonates and adults to different doses of aflatoxin B1, a human hepatocarcinogen that requires a metabolic activation;
  3) Determine the effect of exposure of neonatal and adult Big Blue mice to 17 ß-estradiol, a human hormone carcinogen, on subsequent spontaneous and carcinogen-induced mutations in the cII gene of the target organs; and,
  4) Determine the types of cII mutations in the mutants from Obj. 1, 2, and 3.

- **DNA Adduct Formation, Mutations and Patterns of Gene Expression in Big Blue Rats Treated with the Botanical Carcinogens Riddelliine, Aristolochic Acid and Comfrey** — **E0710001** — **Predictive Toxicology**

  Objective(s):

  1) Treat Big Blue rats subchronically with riddelliine, AA, and comfrey using procedures appropriate for tumor induction;
  2) Analyze DNA adduct formation in the target tissues for carcinogenesis and in spleen lymphocytes;
  3) Determine the cII mutant frequencies and the types of cII mutations in the target tissues of treated rats;
  4) Determine global gene expression patterns in the target and surrogate tissues of treated rats; and,
  5) Correlate gene expression patterns with DNA addcut formation and mutation induction in treated rats.

Title	Project Number	Strategic Research Goal

PI: Dobrovolsky, Vasily

- **Validation of the Mouse Targeted tk+/- *In vivo* System for Use in Mutagenicity Studies** — **E0701801** — **Predictive Toxicology**

  Objective(s):

  1) Expand a colony of transgenic tk+/- mice using breeding of tk+/- founders and C57Bl/6 mice, and transfer the tk+/- genotype to a C57Bl/6 background;
  2) Determine spontaneous mutant frequencies at the tk and hprt loci of splenic T-lymphocytes for mice of different ages;
  3) Induce mutations in tk+/- transgenic mice using treatment with the point mutagen ENU and the clastogens BLM and y-radiation, and measure the kinetics of mutant induction at the tk and hprt loci;
  4) Breed transgenic tk+/- parents in an attempt to derive tk-/- knockout mice, and study the biological significance of the tk gene in mice; and,
  5) Determine how the tk-/- genotype may affect mutant frequencies at the hprt locus.

- **ADDEND: Validation of the Mouse Targeted Tk+/- *in vivo* System for Use in Mutagenicity Studies** — **E0701821** — **Predictive Toxicology**

  Objective(s):

  Propose to breed Tk+/- mice with Pms2+/- mice in order to derive Tk+/- mice that can be used for evaluating LOH mutation and that are also deficient in the Pms2 gene product. Use animals bred under the parent protocol E07018.01 and another protocol (E07041.01). Also extend proposed completion date of master project and associated addenda to 4/30/2004.

- **Transgenic Mouse Model for Detecting *in vivo* Mutation Using a Green Fluorescent Protein Reporter** — **E0713801** — **Predictive Toxicology**

  Objective(s):

  1) Produce two lines of transgenic mice expressing the tetracycline-repressor protein;
  2) Investigate the efficiency of *in vivo* repression of green fluorescent protein (GFP) in various tissues of different lines of the double-transgenic mice; and,
  3) Determine the frequency of spontaneous and y-ray-induced TetR mutation in lymphocytes of double-transgenic mice using flow cytometry.

Title	Project Number	Strategic Research Goal

PI: Fuscoe, James

- **Development of Glass-slide Based Oligonucleotide Method-Driven Microarrays for Mouse and Human Genes** — **E0711601**

  Objective(s):

  Develop, print, and establish the methodology for using a "mouse chip" containing approximately 5000 genes, a "rat chip" containing approximately 4000 genes, and a "human chip" containing approximately 8300 genes. Once the methodology is established, these chips will be available for a wide variety of research projects within the NCTR.

- **General Support for Center for Functional Genomics** — **S00616** — **Predictive Toxicology**

  Objective(s):

  The Center for Functional Genomics (CFG) is a centralized facility to handle all aspects of microarray printing and processing. Objectives are to:

  1) Provide NCTR investigators with access to high quality microarray technology for the investigation of biological mechanisms of action underlying the toxicity of products regulated by the FDA, and related fundamental and applied research;
  2) Create a validated toxicogenomics database that will be a resource for the scientific and regulatory community;
  3) Be a focal point and scientific resource for issues in toxicogenomics; and,
  4) Utilize advances in genomics to address issues critical to the FDA mission. In addition, the CFG will provide continual development of new and better approaches to microarray technologies, including larger gene collections, custom microarrays, validated gene expression databases, experimental design, and tools for handling and analyzing microarray data.

Title	Project Number	Strategic Research Goal

PI: Hansen, Deborah

- **Mechanism(s) of Folate-Responsive Dysgenesis** **E0707401** **Concept-Driven**

  Objective(s):

  1) Determine if there is concordance between the expression of the folate receptor (FBPI) and the most proliferative cohorts of neural tube- and neural crest-cells during defined 12-hr windows on each day of gestation from GD-5 to GD-15, and determine if the loss of these cohorts of cells during these windows of antifolate exposure give rise to recognizable neural tube defects and neurocristopathies in the fetus at term;
  2) Characterize the basal expression of FBPI isoforms and extent and mechanism of FBPI regulation in the placenta and various fetal tissues on GD-17 among cohorts of dams fed a folate-deficient or folate-replete diet;
  3) Determine if sustained quenching of placental cytotrophoblast FBPI by antisense FBPI cDNA overexpression from GD-8 to GD-16 during maternal folate deficiency has an adverse impact on cytotrophoblastic proliferation leading to small placentas and global growth retardation of fetuses; and,
  4) Demonstrate that neural tube closure and neural crest cell function in the whole mouse embyro at GD-8.5 can be perturbed by down-regulating FBPI expression in neural tube cells through the introduction of antisense oligonucleotides to the 43-kDa trans-factor which is required for FBPI transcription.

- **Examination of Embryonic Gene Expression during Neural Tube Closure** **E0710901** **Concept-Driven**

  Objective(s):

  1) Construct serial analysis of gene expression (SAGE) library of expressed genes from control untreated gestation day 8.0 and GD-8.25 CD-1 mouse embryos;
  2) Construct SAGE library of expressed genes from GD-3.25 CD-1 mouse embryos treated with a teratogenic dose of valproic acid on GD-8;
  3) Compare the libraries to determine which genes are up- or down-regulated by valproic acid treatment;
  4) Use Northern blot techniques to determine of the mRNA transcripts for these genes are indeed increased or decreased in expression compared to control embryos;
  5) Use Northern blot techniques to determine a time-course of altered gene expression for genes of interest;
  6) Examine expression of some of these genes after treatment with teratogenic or non-teratogenic doses of valproic acid, valproate analogs or another developmental toxicant; and,
  7) Use *in situ* hybridization, laser capture microdissection and Northern techniques to determine if altered gene expression is specific for subsets of embryonic cells.

Title	Project Number	Strategic Research Goal

- **Mechanism of Biotin Deficiency-induced Mal-formations** — **E0713301** — **Concept-Driven**

Objective(s):

1) Determine if palatal tissue from biotin-deficient embryos is able to fuse *in vitro* in either biotin-sufficient or -deficient medium;
2) Determine if arachidonic acid increases palatal fusion and improved limb development and increases the length of the long bones *in vitro* from biotin-deficient mouse embryo;
3) Determine if prostaglandin E2 increases palatal fusion and improved limb development and increases the length of the long bones *in vitro* from biotin-deficient mouse embryos;
4) Determine if malonyl CoA increases palatal fusion and improves limb development and increases the length of the long bones *in vitro* from biotin-deficient mouse embryos;
5) Determine fetal arachidonic acid content and synthesis *in vivo*; and,
6) Determine if arachidonic acid is able to prevent biotin deficiency-induced orofacial clefting and limb hypoplasia *in vivo.*

PI: Hass, Bruce

- **Identification of Target Sites for UVB Irradiation in Gene A of F X174 contained as a Transgene in Mouse Embryonic Cell PX-2** — **E0710101** — **Predictive Toxicology**

Objective(s):

1) Determine the dose-survival response of PX02 cells to UVB/UVA light in order to determine UV doses that optimize mutation induction and cell survival;
2) Determine the induced mutant frequency in gene A of FX174 by a forward mutation assay using cultures of PX2 exposed to UVB; and,
3) Sequence the UVB/UVA-induced mutants from treated and untreated cultures to identify specific target sequences.

PI: Heflich, Robert

- **ADDEND: Micronucleus and Gene Mutation Analysis in Female Big Blue B6C3F1 Mice Administered Malachite Green and Leucomalachite Green in the Diet (Addend to E0212821)** — **E0212841** — **Agent-Driven**

Objective(s):

Assess the mutagenicity of malachite green and leucomalachite green in relation to DNA adduct formation in female Big Blue mice.

Title	Project Number	Strategic Research Goal

- **Effect of Azathioprine in Somatic Cell and Germline Hprt Mutant Frequencies in the Mouse** — E0709901 — Concept-Driven

  Objective(s):

  Test the hypothesis that *in vivo* selection by azathioprine affects both somatic cell and germline Hprt mutant frequencies using the mouse.

PI: MacGregor, James

- **An Efficient Regulatory Method for Evaluating Chromosomal Damage** — E0714001 — Method-Driven

  Objective(s):

  A collaborative project to evaluate a new method for monitoring chromosomal damage, involving NCTR, CDER, CFSAN, CVM and Litron Laboratories has been initiated. Flow cytometric scoring of micronucleated cells in peripheral blood samples is being compared with traditional microscopic scoring, and the kinetics of micronucleated cell appearance and disappearance is being determined in species of regulatory interest (rat, dog, non-human primate, human). It is expected that the new methodology, by allowing measurement in peripheral blood rather than bone marrow, will permit integration of studies of chromosomal damage into routine toxicological studies and will facilitate evaluation of chromosomal damage in human studies.

PI: Manjanatha, Mugimane

- **ADDEND: Micronucleus and Gene Mutation Analysis in F344 Big Blue Rats Administered Leucomalachite Green in the Diet for 4, 16, and 32 weeks** — E0212821 — Agent-Driven

  Objective(s):

  Malachite and leucomalachite green are currently being tested for carcinogenicity under the NIEHS/NCTR IAG. Previous experiments indicate that both compounds form DNA adducts in rodents when administered in the diet. The objective of this project is to assess the mutagenicity of leucomalachite green in relation to DNA adduct formation in tissues of Big Blue rats.

Title	Project Number	Strategic Research Goal

- **ADDENDUM: Micronucleus and gene mutation analysis in F344 Big Blue rats administered leucomalachite green in the diet for 4, 16, and 32 weeks. (Addendum 3 to E0212801)** — E0212831 — Agent-Driven

  Objective(s):

  Malachite and leucomalachite green are currently being tested for carcinogenicity under the NIEHS/NCTR IAG. Recent results from addendum 2128.21, indicate a two-fold increase in lacl mutations in the livers of Big Blue rats fed leucomalachite green for 16 weeks. The objective of this addendum is to expand the analyses of the remaining rats on 2128.21 (32-week dose groups) to include additional indicators of hepatic toxicity.

PI: Mckinzie, Page

- **Application of the MutEx/ACP-PCR Method of Genotypic Selection to the Detection of K-ras Mutations** — E0706601 — Predictive Toxicology

  Objective(s):

  Establish assays that can provide mechanistic data for chemical risk assessment and aid in establishing the relevance of rodent models for predicting human risk. The proposed research approach is to apply a recently developed method, MutEx/ACP=-PCR to the detection of human and rodent k-ras GGT ->GAT and GGT -> GTT mutations. The assays will then be used to study the chemical induction of these mutations.

PI: Morris, Suzanne

- **ILSI/HESI Consortium on Application of Genomics and Proteomics to Mechanism-Based Risk Assessment** — P00425 — Knowledge Base

  Objective(s):

  The goal of the ILSI project is to:

  1) Establish a database for genomics data; and,
  2) Relate the changes in gene expression to *in vitro* genotoxicity measures that are utilized in hazard assessment. In this project, two cell strains will be exposed to known carcinogens, the mutant frequency at the Thymidine Kinase locus will be measured and the formation of specific DNA adducts will be quantified. RNA will be isolated and sent to CDER for gene expression analysis. The data generated from this project will be entered into the ILSI database for further analysis.

Title	Project Number	Strategic Research Goal

PI: Parsons, Barbara

- **Measurement of H-ras Codon 61 CAA AAA Mutation in Mouse Liver DNAs using the MutEx/ACB-PCR Genotypic Selection** — **E0704101** — **Predictive Toxicology**

  Objective(s):

  1) Quantify somatic mutations in liver DNA of mice treated with 4-aminobiphenyl in order to establish and evaluate MutEx/ACB-PCR genotypic selection as an approach for human risk assessment; and,
  2) Determine whether or not the MutEx/ACB-PCR genotypic selection is sensitive enough to measure the spontaneous frequencies of H-ras codon 61 CAA AAA mutation in three different mouse models: B6C3F1, C57BL/6, and the Pms2 mismatch repair-deficient, transgenic mouse.

- **ADDEND: Measurement of H-ras Codon 61 CAA AAA Mutation in Mouse Liver DNAs using the MutEx/ACB-PCR Genotypic Selection** — **E0704121** — **Predictive Toxicology**

  Objective(s):

  Quantify and identify lacI mutations in liver DNA of mice treated as neonates with 4-aminobiphenyl in order to establish mutation induction and specificity as an early event in hepatic tumorigenesis.

- **Pms2 Mismatch Repair-Deficient Mouse Breeding Colony** — **S00601** — **Predictive Toxicology**

  Objective(s):

  The objective is to maintain a breeding colony of Pms2 transgenic mice, which are not available commercially, so this strain can be used in future protocols. Because these animals are mismatch repair-deficient, they accumulate mutation to a greater extent than their mismatch repair-proficient counterparts and therefore, are a valuable mouse model for mutation research. Heterozygous Pms2 +/- animals will be maintained and as protocols are developed, we will have the capacity to breed the necessary Pms2 +/-, mismatch repair-deficient animals.

Title	Project Number	Strategic Research Goal

PI: Valentine, Carrie

- **Evaluation of the Potential of the Gene A Forward Mutational Assay of PhiX174 for Improving Sensitivity of Transgenic Mutation Assays** — E0711501 — Predictive Toxicology

  Objective(s):

  1) Determine the appropriate experimental conditions to identify single bursts of mutations fixed *in vivo*;
  2) Develop a microplate scoring method that will identify *in vivo* bursts within numerous aliquots;
  3) Determine the spontaneous mutant frequency and ENU-induced mutant frequency by single burst analysis for mouse splenic lymphocytes; and,
  4) Continue development of a frameshift assay for phiX174 in gene J by our collaborator Dr. Bentley Fane.

- **Maintenance of Breeding Colony of C57Bl6 Mice Homozygous for the PhiX174 Bacteriophage Transgene** — S00600 — Predictive Toxicology

  Objective(s):

  Maintain a breeding colony of transgenic mice for the purpose of testing this mouse as a new *in vivo* model for genotoxicity testing. The breeding colony will provide homozygous mice to breed with CH3 mice in order to produce C57H3F1, which is the standard NTP mouse. These F1 will be used in future protocols to test compounds other than ENU, which was tested under protocol E06977.01. This S project is requested to maintain the colony from which future protocols will conduct the cross-breeding.

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Concept Papers

Title	Project Number	Strategic Research Goal

PI: Bendre, Sachin

- **DNA Damage and Somatic Cell Mutation in Children Born to Malnourished Mothers** — **E0710701** — **Predictive Toxicology**

Objective(s):

1) Establish populations of well-nourished and malnourished mothers and their offspring by clinical examination and establishing a Body Mass Index for pregnant women and by anthropometry on their newborns;
2) Among the populations of well-nourished and malnourished pregnant women, identify subpopulations whose diets have not been supplemented or have been supplemented through clinical intervention for varying periods and/or doses with iron and folate;
3) Measure the frequency of 6-thioguanine-resistant lymphocytes as a marker of somatic cell mutant frequency in the women and their offspring; and,
4) Explore possible mechanisms for any alterations in the frequency of 6-TG-resistant lymphocytes in the offspring of both supplemented and unsupplemented malnourished mothers.

PI: Chen, Tao

- **Further Evaluation of the Types of Genetic Events Detected by the Mouse Lymphoma Assay (MLA) and the Role of the Assay in Mechanistically Based Risk Assessment** — **E0711701** — **Predictive Toxicology**

Objective(s):

1) Determine if the L5178Y/TK+/- Mouse Lymphoma Assay adequately detects both aneuploidy and mitotic recombination;
2) Determine if the L5178Y mouse lymphoma cells have active recombinase functions which lead to a large proportion of mutants that result from recombinase-mediated rearrangements; and,
3) Determine what is/are the fundamental genetic mechanism(s) causing the small and large colony thymidine kinase mutant phenotypes.

Title	Project Number	Strategic Research Goal

PI: Fuscoe, James

- **Assessment of the Expression of Disease-Associated Susceptibility Genes during the Life Cycle of Rats** — E0712201 — Concept-Driven

Objective(s):

1) Use the NCTR rat microarray chip to quantitate the relative expression of approximately 4000 genes in the liver of rats at the following ages: 2 wks, 5 wks, 6 wks, 8 wks, 15 wks, 21 wks, 52 wks, 78 wks, and 104 wks. These data will serve as a baseline measurement of gene expression that will be available for future studies on drug metabolism, toxicity, and susceptibility; and,
2) Verify the relative expression levels by quantitative PCR or Northern analysis.

PI: Heflich, Robert

- **Rodent Kidney Cell Culture for Use in Evaluating *in vivo* Mutation: A Methods Development Protocol** — E0712501 — Method-Driven

Objective(s):

The specific goal of this methods development protocol is to gain experience in and adapt kidney cell-culturing techniques that will be used for measuring endogenous reporter gene mutant frequencies in rats and mice.

PI: Mckinzie, Page

- **Concept - ACB-PCR Detection of Azoxymethane-induced Rat K-ras codon 12 GGT>GAT and GGT>GTT Mutations in colonic Aberrant Crypt Foci Isolated using Laser-capture Microdissection** — E0714901 — Concept-Driven

Objective(s):

1) Treat F344 rats with subcutaneous azoxymethane at a concentration known to induce tumor formation;
2) Isolate aberrant crypt foci from rat colon using laser-capture microdissection;
3) Determine K-ras codon 12 GGT>GAT and GGT>GTT mutant fraction in the aberrant crypts; and,
4) Determine the correlation between k-ras codon 12 mutant fraction with time post-exposure.

Title	Project Number	Strategic Research Goal
◆ **Development of Techniques for DNA Isolation from Formalin-fixed Archived Tissue using Laser Capture Microdissection Suitable for Subsequent Allele-specific Competitive Blocker PCR**	**P00613**	**Method-Driven**

Objective(s):

Allow training of the investigator in the use of the LCM techniques and determine a working protocol for using LCM to isolate cells from archived tissue for the purpose of performing ACP-PCR studies.

PI: Parsons, Barbara

◆ **Concept - Analysis of p53 Codon 270 CGT->TGT Mutation in Simulated Solar Light-induced Skin Tumors and Exposed Mouse Skin**	**E0715201**	**Predictive Toxicology**

Objective(s):

1) Develop the ACB-PCR detection of mouse p53 codon 270 CGT->TGT mutation;
2) Measure the frequency of detection and levels of this mutation in mouse skin tumors;
3) Measure the frequency of this mutation in skin tissue from tumor-bearing animals; and,
4) Measure the frequency of this mutation in skin exposed to decreasing levels of SSL.

Microbiology

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Senior Analyst, Don Paine, examines microscopically a sample for the presence of parasites.

Executive Summary

Introduction

The Division of Microbiology at the NCTR serves a multipurpose function with specialized expertise to perform fundamental and applied research in microbiology in areas of the FDA's responsibility in toxicology. The Division of Microbiology also responds to microbial surveillance and diagnostic needs for research projects within the NCTR and FDA. Projects are selected based on FDA priorities and programmatic expertise. The research program is divided into six focal areas: 1) foodborne pathogens, food safety and methods development; 2) antimicrobial resistance; 3) gastrointestinal microbiology and host interactions; 4) environmental biotechnology; 5) use of microorganisms as models to predict the metabolic pathways by which drugs are metabolized in mammals; and 6) microbiological surveillance and diagnostic support of research.

FY 2002 Accomplishments

The Division of Microbiology research scientists continue to provide valuable information to FDA on evaluating key regulatory issues in food safety and environmental biotechnology, with special emphasis on antimicrobial resistance in the food animal production environment.

In a collaborative project with scientists at the Center for Food Safety and Applied Nutrition (CFSAN), Dauphin Island laboratory, investigators in the Division of Microbiology developed a polymerase chain reaction-based method to specifically identify *Vibrio parahaemolyticus* 03:K6 isolates. This method will now be used to fingerprint *V. parahaemolyticus* isolates from seafood and environmental samples. Scientists in the Division of Microbiology have also collaborated with investigators in the Division of Chemistry on the rapid identification of bacteria by mass spectrometry.

Reports of antimicrobial-resistant bacteria from farms and animal carcasses and aquaculture facilities are raising concerns that antimicrobial use in food-producing animals may play a role in selecting for antibiotic resistance. The research and regulatory issues on antimicrobials used in food-producing animals are of great importance to the FDA. A number of collaborative research projects with other FDA centers are being conducted in the Division of Microbiology.

Project Number Codes:
E–Ongoing P–Preliminary S–Support

Researchers in the Division of Microbiology have collected litter, feed and water samples from farms to isolate *Salmonella*, *Campylobacter*, and *Escherichia coli* to determine if they are fluoroquinolone-resistant. Molecular methods, such as ribotyping, pulsed field gel electrophoresis and polymerase chain reaction, were developed to screen for fluoroquinolone resistance genes in *Salmonella* spp., *Campylobacter* spp. and *E. coli* isolates from chicken and turkey farms. Molecular characterization of the fluoroquinolone-resistant strains was conducted. In addition, several tetracycline-resistant bacteria were isolated from samples obtained in aquaculture facilities.

Since there has been concern about the use of antibiotics in agriculture, other approaches are being evaluated to minimize contamination of animal products with foodborne human pathogens. Reducing colonization of animals by pathogenic bacteria by using competitive exclusion treatments is being considered as an alternative to antimicrobial feed additives.

Competitive exclusion products must adhere to FDA regulations that the bacterial mixtures be well-defined, pathogen-free, not resistant to antimicrobials, and effective. For commercial use, competitive exclusion preparations for poultry must be free from all known human and avian pathogens and from any microorganisms with unusually high resistance to antimicrobials. The FDA has approved a competitive exclusion product designed to prevent the colonization of chicken intestines by pathogenic bacteria, such as *Salmonella* spp., *Campylobacter* spp., and *E. coli*, and also to reduce the use of antimicrobials and the spread of antimicrobial-resistance genes. Researchers in the Division of Microbiology have standardized a quick and accurate *in vitro* assay for determining the efficacy of potential competitive exclusion products to protect against *Salmonella* invasion. In addition, researchers have characterized vancomycin-resistant isolates from a competitive exclusion product. Our studies provide the FDA with methods, which will help to standardize the identification techniques used to characterize the components of competitive exclusion products.

Intestinal microflora play significant roles in human health because they aid in the digestion of food, metabolize drugs and foreign compounds, and help prevent pathogens from colonizing the gastrointestinal tract. In response to FDA's need for assessing the microbiological safety of animal drug residues in food, the Division of Microbiology and CVM have been performing pre-validation studies on an *in vitro* system that examines the effect of low-level antibiotic residues on the human intestinal microflora by using a continuous culture to model the human intestinal tract. In FY 2002, the *in vitro* continuous culture system for the analysis of low levels of antimicrobials on the human intestinal microflora was modified and refined. Recommendations on the methods and protocols for determining the effect of residual levels of antimicrobials on the human intestinal microflora were presented at several meetings of the Microbial Safety Task Force of the Veterinary International Cooperation and Harmonization Safety Working Group. Guidance documents have been drafted on determining the effect of residual levels of antimicrobials on the human intestinal microflora.

The development and evaluation of oligonucleotide-microarray and DNA membrane array methods to rapidly detect human intestinal microflora population changes following exposure to xenobiotics were conducted. The utility of these methods for testing the impact of antimicrobial residues, food additives and probiotic products is currently being investigated.

Another essential study in the Division of Microbiology is the elucidation of the mechanism of resistance to antimicrobial agents among bacteria from the human gastrointestinal tract. The resistant bacteria are of particular concern, because not only do they act as a reservoir for antimicrobial resistance genes, but also if they establish themselves in other parts of the body, they can cause diseases that cannot be treated. The Division of Microbiology research scientists have detected anaerobic bacteria from the human intestinal tract that are resistant to high concentrations of second generation fluoroquinolones. They also determined that one of the mechanisms of fluoroquinolone-resistance may be due to the bacteria having an active efflux pump, which reduces uptake of the antimicrobial into the cell. The level of expression of the efflux proteins is currently being evaluated.

The environmental fate of veterinary drugs and the factors that influence the persistence and biodegradation of antibiotics used in farm animals and aquaculture have been investigated. Both fundamental and applied studies on the biodegradation pathways of erythromycin and the fluoroquinolones, ciprofloxacin, norfloxacin, and sarafloxacin have been conducted. These studies indicate that microorganisms may play an important role in the detoxification and removal of antimicrobials from animal wastes and aquaculture sites.

Polycyclic aromatic hydrocarbons (PAHs) constitute a class of organic compounds whose environmental fate is of concern because some PAHs have mutagenic, ecotoxic and carcinogenic potential. Scientists in the Division of Microbiology have elucidated the biodegradative pathways and the enzymes involved in PAH metabolism. Proteomic and genomic techniques have been developed to characterize protein expression and the genes involved in the bacterial metabolism of PAHs.

Another ongoing research initiative within the Division of Microbiology is to exploit the use of microorganisms as models of mammalian drug metabolism. Studies were completed on determining that the fungus *Cunninghamella elegans* mimics mammalian metabolism of antidepressant drugs, such as mirtazapine. Microbial metabolites of a wide range of drugs can be produced more cost-effectively and in less time than those produced by experimental animals, cell cultures or mammalian enzyme systems for structural elucidation and toxicity evaluation. We have also purified and characterized the cytochrome P-450 and glutathione-*S*-transferases from *C. elegans.*

The primary mission of the Surveillance/Diagnostic Program in the Division of Microbiology is to assure that the experimental animals at NCTR are healthy and free from infections that could compromise research data. They also provide researchers critical support in microbial culture identification, contamination investigation, stock culture maintenance, media preparation, and technical assistance. A major initiative in FY-2002 was to develop molecular biology detection procedures for each of the microorganisms in our potential animal pathogen list and incorporate these methods into our surveillance screening.

FY 2003 Plans

Work will continue on a number of ongoing projects including:

1) The Importance of Human Intestinal Microflora in Conversion of Phytoestrogens to Estrogenic Compounds – The phytoestrogen metabolites produced by colonic bacteria of different individuals and the individual bacteria present in the colonic bacteria of the positive samples isolated and further incubated with daidzein for the detection of specific bacteria involved in daidzein metabolism;
2) Studies on Mechanism of Fluoroquinolones Resistant *Salmonella* spp. Isolated from Animal Feeds (Poultry), Animal Production and the Development of Molecular Methods for Screening the Drug Resistance Genes – characterize *E. coli, Salmonella* and *Campylobacter* strains from turkey and chicken samples using a variety of molecular biology methods and study the effects of environmental enteric pathogens on intracellular signaling of the host;
3) Studies on the Fluoroquinolone Resistance in *Campylobacter* sp. Isolated from Poultry - characterization of campylobacters from turkey litter, and data on the correlation of environmental factors on the occurrence of campylobacters in turkey and chicken farms will be conducted;
4) Microbial Models for Biotransformation of Fluoroquinolones – identify the transformation products that are produced by *Pestalotiopsis guepini* from enrofloxacin, ofloxacin, and fleroxacin;
5) *In vitro* Assay for Perturbation of Colonization Resistance by Antibiotic Residues – test the effects of antimicrobial agents on the ability of the new model human intestinal microflora to protect against *Salmonella* and *Campylobacter* sp. invasion of Caco-2 cells and test a new human intestinal cell line in the assay;
6) Determining the Effect of Low Levels of Antibiotic Residues on the Human Intestinal Microflora using an *in vitro* Continuous Culture System - sequence the mutated *gyrA* region of the ciprofloxacin-resistant *E. coli* from the continuous culture system and compare with the isolates from the fecal inocula, to determine if the appearance of the resistance was due to amplification of existing organisms. In addition, conduct investigations on the utility of DNA membrane arrays or microarrays for monitoring continuous or batch intestinal microflora cultures;
7) Elucidation of the Mechanism of Resistance Development in Anaerobic Bacteria from the Human Intestinal Tract – Evaluate the level of expression of the efflux protein in the presence and absence of fluoroquinolones and characterize and purify the cloned protein;
8) Probiotic Effects on Host Defense Against Enteric Pathogens – Acquire germfree mice and colonize them with the model intestinal microflora and evaluate the colonization of the mice by the intestinal microflora;
9) Proteomic Approaches to Elucidate Biodegradative Pathways – Identify differentially expressed proteins by N-terminal sequencing and mass spectrometry and determine condition-specific marker proteins, which are part of the response of the bacteria under different conditions. Furthermore, we will elucidate the metabolic pathways for benzo[*a*]pyrene, benz[*a*]anthracene and dimethylbenz[*a*]anthracene in *Mycobacterium* PYR-1 and characterize the PAH degradative genes using molecular techniques;
10) Novel Molecular Approaches for the Detection and Analysis of the Predominant Bacterial Species in the Human Gastrointestinal Tract – Design and evaluate microarray-slides to detect 40 intestinal bacterial species from human fecal samples.

In addition, two new projects will be developed: Development of Molecular Methods including Oligo-Microarray Methods for the Detection and Monitoring of Foodborne Pathogenic Bacteria and Development of a microarray chip for the detection of multiple antibiotic resistance markers. We will also continue to collaborate with investigators in the Division of Chemistry on the rapid identification of bacteria by mass spectrometry.

Public Health Significance

The FDA, various national and international committees, and the general public are concerned about the increased multiple antimicrobial resistance that recently has been found among pathogenic microorganisms. This may be due in part to the veterinary use of antimicrobials, which will potentially bring about a general increase in the numbers of antimicrobial-resistant bacteria in food animals and the environment and increased amounts of antimicrobials and their biotransformation products in meat, milk or egg products that could affect consumers via the intestinal microflora. The issue of microbial drug resistance has significance both to health and regulatory agencies. The FDA has expressed an interest in research that would determine whether antimicrobial resistance occurs in bacteria isolated from animal feeds containing antibiotics, the pattern of resistance development in bacteria found in animals fed antibiotics, and differences in survival rates of drug-resistant pathogens compared to non-resistant pathogens. Various antimicrobial drugs are currently approved for growth promotion in food animals by Canada, Mexico, Australia, New Zealand, and the European Union as well as the United States. The Division of Microbiology has taken a multi-disciplinary approach to provide fundamental information to the FDA on antimicrobial resistance, environmental biotechnology, and food safety issues.

Research Projects

Title	Project Number	Strategic Research Goal

PI: Campbell, Warren

- **Animal Husbandry Breeding Support** **E0002200** **Center Support**

  Objective(s):

  Microbiological evaluation of animals and non-animal samples not specifically designated to an ongoing experiment.

- **Conventional Mice Breeding** **E0010900** **Center Support**

  Objective(s):

  Determine health status of mice breeding colonies maintained under conventional conditions.

- **SPF Rat Breeding Colony** **E0011000** **Center Support**

  Objective(s):

  Determine health status of rat breeding colonies maintained under specified pathogen free conditions.

- **Conventional Rat Breeding Colony** **E0011100** **Center Support**

  Objective(s):

  Determine health status of rat breeding colonies maintained under conventional conditions.

- **Conventional Guinea Pig Breeding Colony** **E0011200** **Center Support**

  Objective(s):

  Determine health status of guinea pig colonies maintained under conventional conditions.

- **Quarantine Animals** **E0011300** **Center Support**

  Objective(s):

  Determine health status of animals received at NCTR and held under quarantine conditions.

- **Diet Prep General Support** **E0014500** **Center Support**

  Objective(s):

  Determine the microbial contamination level in dosed or control feed and water lots prepared for animal use but not designated to a specific ongoing experiment.

- **Primate Colony Surveillance** **E0023500** **Center Support**

  Objective(s):

  Determine the health status of the primate colonies maintained at NCTR.

Title	Project Number	Strategic Research Goal

- **Microbiological Diagnostic Methods:Development, Testing, & Evaluation** — **E0026200** — **Method-Driven**

  Objective(s):

  Improve diagnostic and epidemiological capabilities in bacteriology, parasitology, mycology, virology and serology as applicable to NCTR programs and projects.

- **General Microbiological Support –Bacteriology, Parasitology, Mycology and Virology** — **S00006** — **Center Support**

  Objective(s):

  Determine health status of animal colonies and their environment.

- **Microbiology Division – Media Preparation** — **S00064** — **Center Support**

  Objective(s):

  Provide media and reagent preparations to both research and surveillance/diagnostic needs.

- **Special Epidemiology Investigations of Potential Microbiological Contamination Problems** — **S00185** — **Center Support**

  Objective(s):

  1) Investigate potential microbiological contamination problems; and,
  2) Report non-routine sample time which is not recorded on Sample Collection Report (SCR).

PI: Cerniglia, Carl

- **Proteomic Approaches to Elucidate Biodegradative Pathways** — **E0711801** — **Method-Driven**

  Objective(s):

  1) Use proteomic approaches to isolate putative catabolic proteins that are over-expressed when microorganisms are grown in the presence of polycyclic aromatic hydrocarbons; and,
  2) Develop software to analyze 2-D gels.

Title	Project Number	Strategic Research Goal

PI: Erickson, Bruce

- **Determining the Effect of Low Levels of Antibiotic Residues on the Human Intestinal Microflora using an *in vitro* Continuous Culture System** — **E0709201** — **Method-Driven**

Objective(s):

Determine the concentration of selected fluoroquinolones that produce no adverse effect on the human intestinal microflora. Hypothesize that an *in vitro* chemostat culture system that mimics the human intestinal tract can be used to detect and characterize the effect of low-level antibiotic residues in food on the human intestinal microflora.

PI: Khan, Ashraf

- **Studies on Mechanism of Fluoroquinolones Resistant Salmonella spp. Isolated from Animal Feeds (Poultry), Animal Production Environment and the Development of Molecular Methods for Screening the Drug Resistance Genes** — **E0704801** — **Method-Driven**

Objective(s):

1) Isolate, identify and characterize nalidixic acid and fluoroquinolone-resistant *Salmonella spp*. from chicken farms (animal feed, feces, manure, litters and animals) by biochemical and Polymerase Chain Reaction;
2) Determine minimum inhibitory concentration for environmental isolates, develop of molecular techniques and compare clinical strains;
3) Determine drug-resistance mechanisms in the environmental isolates and characterize them by molecular techniques; and,
4) Determine the influence of seasons and the frequency of isolation of fluoroquinolone-resistant *Salmonella spp*.

Title	Project Number	Strategic Research Goal

PI: Khan, Saeed

- **Molecular Screening Methods for the Determination of Vancomycin Resistance in Selective Competitive Exclusion Product CF3 (PREEMPT) Bacteria** — E0705301 — Method-Driven

Objective(s):

1) Islolate, identify and biochemically characterize vancomycin-resistant bacteria present in a commercially available competitive exclusion product CF3;
2) Develop a rapid PCR method for the detection of vancomycin-resistance-determinant genes, namely, the Van A0, Van B, Van C and D-ala-D-lac ligase gene D;
3) Characterize plasmid DNA Profile and plasmid-mediated drug resistance transfer;
4) Genetically fingerprint the vancomycin-resistant microorganisms present in PREEMPT culture; and,
5) Nucleotide sequence analysis of the PCR products of vancomycin-resistant determinant genes showing interesting restriction profiles.

PI: Nawaz, Mohamed

- **Studies on the Fluoroquinolone Resistance in Campylobacter sp. Isolated from Poultry** — E0705001 — Method-Driven

Objective(s):

1) Isolate and identify fluoroquinolone-resistant *Campylobacter jejuni* and *C. coli* from water, feed and litter samples in poultry houses;
2) Determine the optimum concentration of nalidixic acid and fluoroquinolone resistance in *C. jejuni* and *C. coli*;
3) Determine the influence of various seasons and the frequency of isolation of fluoroquinolone-resistant *C. jejuni* and *C. coli*; and,
4) Molecularly characterize fluoroquinolone resistance by polymerase chain reaction (PCR), nucleotide sequencing and single-strand conformation polymorphism (SSCP).

Title	Project Number	Strategic Research Goal

- **The Fate and Degradation of Antimicrobials, Oxytetracycline (OTC) and Sulfadimethoxine-Ormetoprim (Romet-30) from Aquaculture Environmental Samples** — **E0707501** — **Method-Driven**

  Objective(s):

  1) Determine the biodegradation rates and metabolic fate of antimicrobials, oxytetracycline and Sulfadimethoxine-Ormetrorpim (Romet-30) (SDO), used in fish farming systems; and,
  2) Isolate, characterize and identify OTC- and SDO-resistant organisms from aquaculture sediment and natural environment samples and conduct molecular characterization of the genes that regulate resistance to the drugs.

PI: Rafii, Fatemeh

- **Importance of Human Intestinal Microflora in Conversion of Phytoestrogens to Estrogenic Compounds** — **E0700701** — **Concept-Driven**

  Objective(s):

  1) Detect various metabolites of phytoestrogens, produced by the metabolism of these compounds by pure culture of bacteria typical of that isolated from human microflora, and elucidation of the metabolic pathways of phytoestrogens by human intestinal bacteria;
  2) Assess the estrogenic effect of each phytoestrogen metabolite produced by intestinal bacteria;
  3) Determine the bacterial species producing estrogenic metabolites from phytoestrogens and elucidate enzymes involved in various steps of these metabolic processes; and,
  4) Determine the effects of phytoestrogens and their metabolites on the population, composition, metabolic activity and enzyme production of bacteria from the human gastrointestinal tract.

- **Elucidation of the Mechanism of Resistance Development in Anaerobic Bacteria from the Human Intestinal Tract** — **E0709301** — **Knowledge Base**

  Objective(s):

  Evaluate the effect of fluoroquinolones on the resistance development in the bacteria from the human intestinal tract and analyze the fluoroquinolone resistance mechanism in anaerobic bacteria from the human intestinal tract.

Title	Project Number	Strategic Research Goal

PI: Sutherland, John

- **Biotransformation of Fluoroquinolones by Fungi** **E0705201** **Method-Driven**

  Objective(s):

  Measure the kinetics of biodegradation of veterinary fluoroquinolone drugs in natural matrices to identify the potential metabolites produced by fungi from fluoroquinolones, and to assess the residual antibacterial activity and potential risks of the metabolites formed from these drugs.

PI: Wagner, Robert

- ***In vitro* Model and Molecular Analysis of Competitive Exclusion Products** **E0704901** **Method-Driven**

  Objective(s):

  1) Evaluate individual component bacteria in a defined competitive exclusion (CE) product for exclusion of enteric pathogens from Caco-2 and CRL-2117 cell monolayers;
  2) Define the antimicrobial susceptibility patterns of the component bacteria using Minimal Inhibitory concentration measurements;
  3) Perform sequence analysis of 16s rRNA Polymerase Chain Reaction (PCR) products from defined culture component bacteria and develop a database containing the sequences for use in subsequent identification of the organisms in undefined CE products; and,
  4) Apply the 16s rRNA sequence analysis procedure to detect and identify effective CE component bacteria in undefined CE products.

- **Measurement of Antimicrobial Drug Concentrations that Inhibit Colonization Resistance** **E0708601** **Method-Driven**

  Objective(s):

  Adapt an enterocyte culture model of colonization resistance by enteric microbial flora against *Salmonella sp*. colonization/invasion to measure concentrations of antimicrobial drugs as food residues that would inhibit the barrier effect of the consumer's intestinal flora.

Title	Project Number	Strategic Research Goal

- **Probiotic Effects on Host Defense Against Enteric Pathogens** — **E0709701** — **Knowledge Base**

  Objective(s):

  1) Establish a model intestinal bacteria population in mice that consists of human intestine-derived bacteria;
  2) Observe the fate of members of the model bacterial population when probiotic bacteria are fed to the mice;
  3) Observe the fate of the probiotic bacteria fed to the human flora-associated mice;
  4) Observe the effects of the human-derived flora on the host protective systems of the immunodeficient and immunocompetent mice;
  5) Observe effects of adding probiotic bacteria to HFA mice on immunodeficient and immunocompetent host protective systems; and,
  6) Observe the roles of model host flora and probiotic bacteria to modulate host protective systems of the immunodeficient and immunocompetent mice from *Salmonella typhimurium*, and *Campylobacter jejuni*.

PI: Wang, Rongfu

- **Novel Molecular Approaches for the Detection and Analysis of the Predominant Bacterial Species in the Human Gastrointestinal Tract** — **E0711901** — **Method-Driven**

  Objective(s):

  1) Develop a rapid method for quantification of intestinal bacteria;
  2) Perform qualitative analysis of the communities for several major genera and discover the species which are non-cultivated;
  3) Isolate and identify the bacterial species from probiotics used for human or animal health; and,
  4) Develop microarray method for the detection of intestinal bacteria.

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Walia, S.K., Shi, L., Khan, A.A. and Joshi, B., Sequence analysis and molecular characterization of a nitrocatechol dioxygenase gene from Pseudomonas putida, *J. Environ. Sci. Health*, 37:379-391. Accepted: 5/15/2002

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Concept Papers

Title	Project Number	Strategic Research Goal
PI: Khan, Saeed		
◆ **Concept - Development of a Microarray Chip for the Detection of Multiple Antibiotic Resistance Markers**	**E0715101**	**Predictive Toxicology**
PI: Wang, Rongfu		
◆ **Concept - Development of Molecular Methods including Oligo-Microarray Methods for the Detection and Monitoring of Foodborne Pathogenic Bacteria**	**E0715401**	**Method-Driven**

Objective(s):

The Oligonucleotide probes for the detection of known antibiotic resistance markers for at least 17 antibiotics that are used in animal and poultry farming would be printed on a microarray slide. Such a tool will help FDA to monitor and track the resistance markers and help in the regulatory decision-making process.

Objective(s):

1) Develop an oligo-microarray method for the detection of foodborne pathogens based on 16S rDNA sequences;
2) Develop oligo-microarray methods with multiplexed PCR based on many genes for the detection and genotyping of specific pathogenic bacterial strains; and,
3) Develop and modify PCR methods for the detection of all above pathogens.

Molecular Epidemiology

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Researchers utilizing Transgenomics Wave System for SNP Discovery

Executive Summary

Introduction

The strategic goals of the Division of Molecular Epidemiology are: 1) the identification of genetic polymorphisms that influence drug and carcinogen metabolism, individual cancer susceptibility, and therapeutic drug efficacy; 2) the conduct of epidemiological studies for post-market surveillance of chemical toxicants found in foods, drugs, cosmetics, and medical devices; 3) human exposure biomonitoring and DNA adduct detection; and 4) the operation of a "Structural Genomics Center" for discovery of single nucleotide polymorphisms (SNPs) and its application to human diagnostics.

FY 2002 Accomplishments and FY 2003 Plans

The intent is to: better understand the mechanisms of human carcinogenesis; provide an estimation of human exposure to direct and indirect-acting carcinogens; to assess the importance of inter-individual differences in carcinogen and drug bioactivation, detoxification, or induced changes in gene expression; and suggest intervention strategies for human cancer prevention. Accordingly, our research has provided new knowledge on the identification of subpopulations that are not only more susceptible to chemical carcinogens, but also those that are likely to experience adverse drug reactions or decreased therapeutic drug efficacy. Our research has been focused on the food borne heterocyclic amines, environmental aromatic amines and polycyclic aromatic hydrocarbons, on widely used drugs, as well as on tobacco usage. Projects on the etiology of human cancers of the colon/rectum, pancreas, esophagus, breast, ovary, prostate, lung, urinary bladder, and bone marrow are ongoing. These are outlined as follows:

Studies to identify genetic polymorphisms that influence drug and carcinogen metabolism, individual cancer susceptibility, and therapeutic drug efficacy:

1. Metabolic polymorphisms, DNA repair, and individual cancer susceptibility.
   a) Genetic and epigenetic regulation of cytochrome P450 1A2.
   b) Polymorphisms of cytochrome P450 1B1 and tissue-dependent expression.
   c) Polymorphisms of sulfotransferases.
   d) Polymorphisms of glutathione S-transferases.
   e) Inter-individual variation in DNA repair capacity.
   f) Characterization of peroxidases toward metabolic activation.

g) Gender-specific variation in drug metabolism.

2. Chemoprevention.

   a) Modulation of gene expression by chemopreventive agents and identification of gene targets as surrogate biomarkers of effect (*e.g., COX-2, mdr-2, nFkB, DIA4, MnSOD, ras);* and,
   b) DNA methylation, DNA methyltransferases, methyl donors and cancer risk.

Epidemiology and post-market surveillance for chemical toxicants found in foods, drugs, cosmetics, and medical devices:

1. Etiology of human colorectal cancer: role of dietary heterocyclic amines.
2. Etiology of human breast and prostate cancers in African-Americans and Caucasians.
3. Etiology of human pancreatic cancer: role of carcinogen and drug exposures, chronic pancreatitis, and dietary imbalance.

Human exposure biomonitoring and DNA adduct detection:

Biomarkers of exposure and susceptibility to breast, prostate, esophageal, colon, and urinary bladder cancers.

Research Projects

Title	Project Number	Strategic Research Goal

PI: Ambrosone, Christine

- **Chemical Carcinogenesis: Epithelial Cells in Breast Milk** — **E0697801** — **Predictive Toxicology**

  Objective(s):

  1) Develop and refine a methodology for separation of luminal epithelial cells from human breast milk for DNA extraction;
  2) Detect and quantify aromatic/hydrophobic-DNA adducts in luminal epithelial cells derived from human breast milk;
  3) Detect genetic polymorphisms in carcinogen-metabolizing genes derived from DNA extracted from epithelial cells in human breast milk; and,
  4) Evaluate the relationships between carcinogen-DNA adducts and smoking status, and adduct levels with polymorphisms in NAT1, NAT2, CYP1A1, and GSTM1.

- **ADDEND: Chemical Carcinogenesis: Epithelial Cells in Breast Milk** — **E0697811** — **Predictive Toxicology**

  Objective(s):

  1) Measure the levels of aromatic amines in human breast milk;
  2) Evaluate the mutagenicity of human milk and milk fat in an Ames Salmonella test highly sensitive to aromatic amines; and,
  3) Evaluate relationships between aromatic amines in milk, mutagenicity, and carcinogen-DNA adduct levels in ductal epithelial cells with exposure and susceptibility factors.

- **Prostate Cancer: Exposure, Susceptibility and DNA Adducts** — **E0702101** — **Method-Driven**

  Objective(s):

  1) Determine levels of carcinogen exposure in African-Americans and Caucasians with histologically confirmed prostate cancer using a case-control design;
  2) Evaluate variability in hormone metabolism and susceptibility to carcinogen exposure, as measured by phenotypic and genotypic variability in carcinogen metabolism, and evaluate the interaction of these factors with the exposure data obtained in Objective 1; and,
  3) Characterize DNA adducts in prostate tissue from men with prostate cancer to identify mutagenic agents and evaluate levels of adducts in relation to carcinogen exposure data and susceptibility factors obtained in Objectives 1 and 2.

Title	Project Number	Strategic Research Goal

PI: Chen, Genfu

- **Somatic Alterations in Prostate Cancer and Its Precursor Lesions** — E0711301 — Concept-Driven

  Objective(s):

  1) Test the hypothesis that homoplasmic mutations in mitochondrial genome are elevated in human prostate carcinomas as a consequence of increased oxidative stress;
  2) Test the hypothesis that at least some of the homoplasmic mtDNA mutations detected in prostate carcinomas are also detectable in evolutionarily related precursor lesions identified in the same prostate biopsies;
  3) Test the hypothesis that the incidence and type of homoplasmic mtDNA mutations in benign prostatic hyperplasia differ from those in prostate carcinomas; and,
  4) Test the hypothesis that homoplasmic mtDNA mutations are more sensitive than nuclear markers in delineation of clonal evolution of prostate cancers.

PI: Coles, Brian

- **Dietary Isothiocyanates, Glutathioine S-transferases, and Colorectal Neoplasia** — E0320001 — Concept-Driven

  Objective(s):

  Explore the relationship between dietary isothiocyanates, glutathione S-transferase induction and colon polyp recurrence. NCTR's direct objective is to quantitate glutathione S-transferases in human plasma.

- **ADDEND: Project III: Environmental and Genetic Epidemiology of Colorectal Adenomas** — E0320011 — Concept-Driven

  Objective(s):

  1) Incorporate Project III with respect to the analyses to be performed by NCTR. These analyses are glutathione S-transferase genotype and phenotype as specified in the master project E03200.01, plus MTHFR genotype;
  2) Extend the lifetime of E03200.01 to five years to incorporate lifetime of Project III; and,
  3) Extend the lifetime of the associated CRADA, 300-00-0045, from two to five years to incorporate lifetime of Project III.

Title	Project Number	Strategic Research Goal

PI: Hammons, George

- **Methylation Profile, Gene Expression, and Enzyme Activity of CYP1A2 in Human Livers** — **E0696201** — **Predictive Toxicology**

  Objective(s):

  This protocol will serve as a preliminary study to determine the possible involvement of epigenetic mechanisms in the regulation of the expression of the CYP1A2 gene. The methylation status determined for each sample will be correlated with the expression of the CYP1A2 gene and enzyme activity.

PI: Kadlubar, Fred

- **Rapid, Population-based, Screening Methodology for Genetic Polymorphisms in Adverse Drug Metabolizing and/or Cancer-Related Risk Alleles** — **E0300001** — **Predictive Toxicology**

  Objective(s):

  1) Develop and fabricate a bioarray chip or "risk chip" for the analysis of genetic polymorphisms that affect individual cancer or adverse drug risk;
  2) Validate "risk chip" by comparative analyses with standardized methodologies;
  3) Automate methodologies for large population risk assessment using "risk chip" in a robotic work station; and,
  4) Establish NCTR as an alpha test site to introduce "risk chip" screening analysis as rapid and reliable frontline screening methodology for clinical and population-based molecular epidemiological studies.

- **A Case-Control Study of Pancreatic Cancer & Aromatic Amines** — **E0694601** — **Predictive Toxicology**

  Objective(s):

  Measure the associations of aromatic amine exposure and metabolism with the risk of pancreatic cancer. The sources of aromatic and heterocyclic amines to be studied are cigarette smoking and diet; the metabolic capabilities to be studied are acetylator status and N-oxidation status.

- **Role of Acetylation & N-Oxidation in Colorectal Cancer** — **E0694701** — **Predictive Toxicology**

  Objective(s):

  Confirm the initial findings of our pilot study regarding the roles of heterocyclic amine metabolism and exposure as putative risk factors from the diet or the environment. The sources of heterocyclic amines to be studied are cigarette smoking, diet and cooking methods; the metabolic pathways to be studied include heterocyclic amine N-oxidation status and O-acetylation status.

Title	Project Number	Strategic Research Goal

- **Breast Cancer in African-American Women: Method-Driven Metabolic Modification of Dietary and Hormonal Risk Factors** — E0701501

  Objective(s):

  Examine the role of interindividual variability in response to exogenous agents as it may relate to breast cancer risk in African-American women. By evaluating risk associated with exposure to oral contraceptives, hormone replacement therapy, and modification of that risk by genetic variability in their metabolism, the effects of substances regulated by the FDA on breast cancer risk in African-American women may be further elucidated. Additionally, a successful model to increase African-American participation in research studies would greatly assist in future studies related to FDA-regulated substances in African-American populations.

- **ADDEND: The Role of Glutathione S-transferasse genetic Polymorphisms in Breast Cancer Sensitivity to Radio- and Chemotherapy** — E0701511 — **Predictive Toxicology**

  Objective(s):

  1) Determine expression of enzymes (phenotype) in tumor tissue from women who received adjuvant therapy for breast cancer, using biopsy or surgical tissue specimens, using immunohistochemistry, and evaluate associations between phenotypes in tumor tissue and risk of breast cancer recurrence;
  2) Determine inherited GSTM1, GSTT1 and GSTP1 genotypes in normal tissue from these same women, and determine associations of GSTM1, GSTT1 and GSTP1 genotype with phenotype in tumor tissue; and,
  3) Evaluate if GST genotypes predict breast cancer recurrence following treatment, controlling for other factors that may relate to prognosis.

PI: Lyn-Cook, Beverly

- **The Effects of Nicotine and Other Cigarette Components on Normal and Neoplastic Human Pancreatic Cells: The Role of Low Zinc Levels on Ras, mdr-1 Genes Activation and Metabolizing Enzyme Activities as a Possible Risk Factor for Pancreatic Cancer** — E0701701 — **Predictive Toxicology**

  Objective(s):

  Determine the effects of nicotine and other cigarette components on exocrine and endocrine human pancreatic cells *in vitro*. The final objective of this study is to examine ras, mdr-1, CYP1A1 and CYP1A2 expression in normal and neoplastic human pancreatic tissue grouped according to race and sex obtained from a human tissue bank.

Title	Project Number	Strategic Research Goal

- **Mechanistic Actions of Chemopreventive Agents in Pancreatic Cancer** — **E0707601** — **Predictive Toxicology**

  Objective(s):

  Screen a number of agents found in natural products and establish mechanistic data on their potential as anti-cancer agents against pancreatic cancer.

PI: McClure, Gail

- ***In vivo* Modeling of Steroid-mediated Gender Effects in Drug Metabolism** — **E0704301** — **Predictive Toxicology**

  Objective(s):

  1) Characterize the activity of CYP1A2 in female subjects with regard to age, race, phase of the menstrual cycle, pregnancy, oral contraceptive usage, menopause, and HRT;
  2) Characterize the activity of CYP1A2 in male subjects with regard to age;
  3) Measure estradiol, progesterone, testosterone, cortisol, IL-1, IL-6 and IL-10 levels in female and male subjects studied for CYP1A2 activity;
  4) Correlate the activity of CYP1A2 with circulating levels of cytokines and/or circulating levels of steroid hormones; and,
  5) To statistically assess the impact of each of the measured variables on the CYP1A2 phenotype.

- **ADDEND: Part II of *In vivo* Modeling of Steroid-mediated Gender-effects in Drug Metabolism** — **E0704311** — **Predictive Toxicology**

  Objective(s):

  1) Determine the activity of CYP2D6 and 3A4 in female and male subjects with regard to age, race, phase of the menstrual cycle, pregnancy, oral contraceptive usage, menopause, and HRT;
  2) Measure estradiol, progesterone, testosterone, cortisol, IL-1, IL-6 and IL-10 levels in female and male subjects studied for CYP activity;
  3) Correlate the activity of CYP2D6 and 3A4 with circulating levels of cytokines and/or circulating levels of steroid hormones; and,
  4) Assess statistically the impact of each of the measured variables on the CYP2D6 phenotype and CYP3A4 activity level.

Title	Project Number	Strategic Research Goal

PI: Nowell, Susan

- **Sulfotransferase 1A1 (SULT1A1) Genotype and Phenotype in Relation to Efficacy of Tamoxifen Treatment** — **E0714401** — **Predictive Toxicology**

  Objective(s):

  1) Determine whether induction of SULT1A by 4-OH TAM results in an increase in expressed protein and enzymatic activity toward environmental estrogens in tamoxifen-treated breast cancer patients;
  2) Determine the effect of 4-OH TAM on SULT1A1 activity in breast cancer cell lines;
  3) Determine SULT1A1 genotype in tamoxifen-treated women and genotype-phenotype correlations; and,
  4) Archive blood samples, administer the Block 98 Questionnaire, and determine survival data for future studies.

PI: Poirier, Lionel

- **Colorectal Adenoma Study - Task 1** — **E0707101** — **Predictive Toxicology**

  Objective(s):

  Provide analytical support for the analysis of S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) in red blood cell specimens for an intramural study being conducted by the Nutrition Epidemiology Branch, Division of Cancer Epidemiology and Genetics, NCI.

- **ADDEND: Colorectal Adenoma Study (Task 3)** — **E0707121** — **Predictive Toxicology**

  Objective(s):

  Provide analytical support for the analysis of MTHR-specific activity in red blood cell specimens for an intramural study being conducted by the Nutrition Epidemiology Branch, Division of Cancer Epidemiology and Genetics, NCI.

- **ADDEND: Colorectal Adenoma Study - h-ras and k-ras Methylation (Task 4)** — **E0707131** — **Predictive Toxicology**

  Objective(s):

  Provide analytical support for the analysis of h-ras and k-ras methylation in red blood cell specimens for an intramural study being conducted by the Nutrition Epidemiology Branch, Division of Cancer Epidemiology and Genetics, NCI.

Title	Project Number	Strategic Research Goal

◆ **ADDEND: Colorectal Adenoma Study - IGF-1 Hypermethylation (Task 5)** — **E0707141** — **Predictive Toxicology**

Objective(s):

Provide analytical support for the analysis of IGF-1 hypermethylation in red blood cell specimens for an intramural study being conducted by the Nutrition Epidemiology Branch, Division of Cancer Epidemiology and Genetics, NCI.

PI: Tang, Yong

◆ **The Role of Human Cytochrome CYP1B1 in Drug Metabolism and Carcinogenesis** — **E0699001** — **Predictive Toxicology**

Objective(s):

Elucidate the role of human cytochrome P450 1B1 (CYP1B1) in drug metabolism and carcinogenesis. Specific aims are to:

1) Design and develop peptide-specific antibodies against human CYP1B1;
2) Determine the levels of CYP1B1 protein in various human tissues; Evaluate CYP1B1 expression as a biomarker for tumorigenesis;
3) Identify CYP1B1 inducers among the most common drugs and carcinogens;
4) Identify CYP1B1 substrates, including the endogenous steroid hormones, as well as drugs and carcinogens known to be metabolized by the closely related cytochromes P450 IA1 and IA2;
5) Find specific enzyme inhibitors for CYP1B1;
6) develop a sensitive, convenient, and specific assay method for CYP1B1 enzyme activity *in vitro*; and,
7) Evaluate genetic polymorphism(s) for CYP1B1 as an epidemiological marker for cancer risk.

PI: Teitel, Candee

◆ **Chemoprotection of DNA Adducts of 2-Amino-1-methyl-6-phenylimidazo-[4,5-b]pyridine in the Rat** — **E0689401** — **Predictive Toxicology**

Objective(s):

Examine the effect of the glutathione S-transferase inducers, phenethylisothiocyanate, diallyl sulfide (DAS), 5-(2-pyrazinyl)-4-methyl-1,2-dithiol-3-thione (Oltipraz), garlic powder, cabbage powder, 2(3)-tert-butyl-4-hydroxyanisole (BHA), kahweol palmitate, cafestol palmitate, quercetin, tannic acid, a-angelicalactone, Green tea, and ethoxyquin on the metabolism and DNA adduct formation of the foodborne carcinogen, 2-amino-1-methyl-6-phenylimidazo[4,5-b]- pyridine, in the Fischer 344 rat.

Title	Project Number	Strategic Research Goal

PI: Wise, Carolyn

- **Methylation Status and Cancer Risk** — **E0704601** — **Predictive Toxicology**

Objective(s):

Learn whether methylation status, determined by noninvasive procedures, may be a biomarker of cancer risk in humans. The methylation status will be assessed by measurement of SAM, SAH and homocysteine in blood, and of DNA hypomethylation in lymphocytes. Two-thirds of the work will be supported under the terms of an IAG from NCI.

Publications

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Concept Papers

Title	Project Number	Strategic Research Goal

PI: Hammons, George

- **ADDEND: Assessment of Interindividual Variability in Expression of DNA Methyltransferases, DNMT 3a and DNMT 3b, in Liver and Identification of Factors Influencing Expression** — **E0696211** — **Predictive Toxicology**

  Objective(s):

  1) Determine levels of expression of DNMT 3a and DNMT 3b in liver samples from a pool of donors selected according to smoking status, gender, and age; and,
  2) Provide mechanistic data on the regulation of expression of DNMT 3a and DNMT 3b.

PI: Lyn-Cook, Beverly

- **Concept - CYP1 B1 Polymorphisms in Uterine Leiomyomas: Frequency in African-American Women and Response to Therapy** — **P00443** — **Predictive Toxicology**

  Objective(s):

  Determine the frequency of the polymorphic variant and others of cytochrome P450 1B1 in human uterine leiomyomas cases compared with the frequency in patient-matched controls.

PI: McClure, Gail

- **Chemical Carcinogens: DNA-Adducts in Breast Epithelial Cells** — **E0714801** — **Predictive Toxicology**

  Objective(s):

  1) Develop and refine methodology for separation of luminal epithelial cells from samples obtained from the ductal lavage procedure for use in DNA extraction;
  2) Characterize DNA-adducts in breast tissue from women at high risk for breast cancer undergoing ductal lavage to identify dominant mutagenic agents;
  3) Characterize the most common types of recent exogenous carcinogen exposure in high-risk patients receiving ductal lavage;
  4) Evaluate variability in metabolism and susceptibility to carcinogen exposure, as measured by phenotypic and genotypic variability in carcinogen-metabolizing enzymes, and evaluate the interaction of these factors with the exposure data obtained in Object 2;
  5) Obtain DNA-adduct profiles from ductal lavage samples in women at normal risk for comparison with high-risk women; and,
  6) Compare DNA-adduct profiles with respect to exposure levels and genotypic and phenotypic variability in high-risk and normal-risk women.

Title	Project Number	Strategic Research Goal

PI: Ning, Baitang

- **Discovery and Characterization of Novel Single Nucleotide Polymorphisms in the SULT1A1 Promoter Region** — **C30005** — **Predictive Toxicology**

  Objective(s):

  Scan the 5'-flanking region of the SULT1A1 gene for the presence of single nucleotide polymorphisms using denaturing HPLC. The biological significance of the novel SNPs will also be characterized.

PI: Ratnasinghe, Luke

- **Development of Breast, Lung and Esophogeal Cancer Proteomics and Genomic Signatures for Cancer Early Detection** — **E0715501** — **Predictive Toxicology**

  Objective(s):

  Develop breast, lung and esophageal cancer proteomic and genomic signatures for cancer early detection with the use of plasma pooling and high-resolution Multi-Dimensional proteome analyses and mass-spectrometry-based pooled genomic DNA analyses.

- **Influence of Polymorpisms in Enzymes Involved in Carcinogen Detoxification and DNA Repair on Lung Cancer Risk and Survival** — **E0715701** — **Predictive Toxicology**

  Objective(s):

  Examine the association between the codon 198 polymorphism of hGPX1, and other important polymorphisms and lung cancer risk. Also propose to evaluate the influence of polymorphisms on lung cancer survival post cancer diagnosis.

Neurotoxicology

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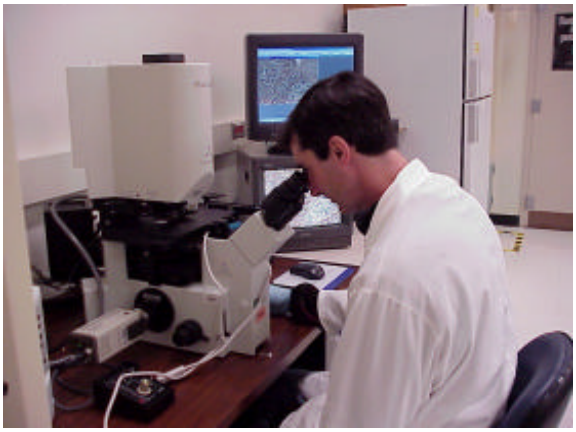

The laser capture microdissection instrument can be used to collect individual cells from tissues sections. Then the cells can be analyzed for genomic and proteomic alterations.

Executive Summary

Introduction

In the United States, brain-related disorders account for more hospitalizations than any other major disease group. One out of four Americans will suffer from a brain-related disorder during their life and the estimated annual cost to the national economy for treatment, rehabilitation, and related consequences is in excess of $400 billion. At no time in the past, however, have researchers been better poised to increase our understanding of brain-related disorders and reduce the risks associated with neurotoxic events.

According to a report from the Congressional Office of Technology Assessment, "Neurotoxicity: Identifying and Controlling Poisons of the Nervous System," the known or suspected causes of brain-related disorders include exposures to chemicals such as therapeutic drugs, food additives, foods, cosmetic ingredients, pesticides, and naturally occurring substances. The number of potential neurotoxicants that require FDA regulation is estimated to be in the thousands and yet guidelines for neurotoxicity risk assessment remain vague and underdeveloped compared to those for cancer. Chemicals from the categories listed above are vital to the national economy and our quality of life. The challenge is to determine at what dose and under what conditions a specific chemical may produce nervous system-related toxicity.

The overall goals of the Division of Neurotoxicology are to develop and validate quantitative biomarkers and precursor events of neurotoxicity and to use these to elucidate modes of action. This will increase the certainty of assumptions underlying human risk assessments for neurotoxicants. The strategy for achieving these goals has been to develop a multidisciplinary approach integrating neurochemical/neurobiological (including genomics and proteomics), neuropathological, neurophysiological, and behavioral assessments to determine adverse effects and explore modes of neurotoxic action. Unique features of the NCTR's neurotoxicology research efforts are the capabilities to determine target-tissue chemical concentrations and cellular level interactions of neurotoxicants and to reduce the uncertainty associated with extrapolating findings across species by effectively using rodent and nonhuman primate animal models--as well as humans--whenever possible.

FY 2002 Accomplishments

Research protocols were developed to provide data in three main focal areas: 1) monoamine (dopamine and serotonin) neurotransmitter systems as a target for neurotoxicity; 2) mitochondrial dysfunction and oxidative stress as mechanisms of neurotoxicity; and 3) the NMDA receptor complex as a mediator of adult and developmental neurotoxicity.

In support of our earlier work on disruption of monoamine neurotransmitter systems, methylenedioxymethamphetamine (MDMA) was shown (as were methamphetamine and fenfluramine before) to produce neuronal cell death in animals that also become hyperthermic as a result of drug treatment. The genomic response to methamphetamine was also systematically described in regional brain areas of the rat. While regional and acute gene expression changes were documented, long-term alterations in gene expression were less robust, suggesting that individual cells collected by laser capture microdissection (LCM) may need to be analyzed to observe the selective effects of these monoaminergic agents. Furthermore, recently reported data suggest that hyperthermia and seizures, as well as stroke, are not necessary for amphetamine to produce neurodegeneration. However, the neurodegeneration that is produced in the absence of these physiological factors is restricted to very discrete areas of the cortex and involves parvalbumin and GABA containing inhibitory neurons, not excitatory pyramidal neurons as might be expected.

In support of our focus on the study of mitochondrial dysfunction and oxidative stress as mechanisms of neurotoxicity, a combination of laser capture microdissection and genomic approaches was developed to identify gene expression profiles associated with aging and mitochondrial dysfunction. This work was conducted in young (3 month-old) and aged (2 year-old) C57 mice. Frozen brain sections were mounted on glass slides and stained cells from selected regions of the hippocampus were collected using LCM. Nuclear extracts containing transcription factors (TFs) that are thought to control levels of oxidative stress, apoptotic pathways and to impact memory were prepared from these cells. TF/DNA complexes were isolated and DNA was then analyzed with a Protein/DNA array that contained cDNA for 54 transcription factors. This approach allows us to test the hypothesis that age-related memory degradation in the hippocampus is associated with age-related changes in gene expression affecting mitochondrial function, apoptosis and levels of oxidative stress. In related studies, the mitochondrial toxicant, 3-nitropropionic acid (3-NPA), was used to test the hypothesis that an enhancer of mitochondrial metabolism, L-carnitine, can also normalize a related set of biomarkers of neurotoxicity including the activity of free radical scavenging enzymes and hypothermia induced by 3-NPA.

Several studies were completed on the role of the NMDA receptor complex as a mediator of adult and developmental neurotoxicity. In the adolescent monkey, it was demonstrated that, in the absence of any “typical” signs of toxicity (i.e., negative findings for clinical chemistries, hematologies, ophthalmic status; home cage and other overt behaviors), exposure to NMDA antagonists can cause severe, long-lasting deficits in cognitive function as measured by performance of a specific learning task. Clearly, such an effect would not be observed under typical toxicity testing protocols. In collaboration with CDER staff, a review article was written and published that provided a review of the literature indicating that administration of ketamine

and other NMDA antagonists during the brain growth spurt results in wide-spread neuronal apoptosis in the rat. The need to provide confirmatory evidence in another animal model more closely resembling the developing human was documented.

FY 2003 Plans

Work will continue on these three focal areas in the coming year. A recently approved protocol will allow us to expand our work in the monoamine neurotransmitter system focal area and address the extent to which the disruption of this system is related to Parkinson's disease. This study will utilize both a mouse model and human tissue samples to determine via proteomic analyses which proteins are affected by neurotoxic insults producing Parkinsonism or Parkinson-like symptoms. In another protocol currently under review, the propensity of Accutane (13-cis-retinoic acid) to induce modification of the dopamine system and to increase depression-related symptoms will be evaluated. Validated behavioral assessments will be used to determine the effects of this retinoid in the adult Sprague-Dawley and the 'depression-prone' Flinders rat.

In the mitochondrial dysfunction and oxidative stress focal area, a newly approved protocol will allow us to define the gene expression profile (genomic approach) associated with 3-NPA exposure in the rat. Gene expression specifically related to mitchondrial function will be the focus of this mechanistic-based protocol. The genomic data will be directly compared to already established biomarkers of 3-NPA neurotoxicity including electrophysiological, histopathological, and biochemical endpoints.

The NMDA-mediated excitotoxic response in the adult rat will be used to isolate and characterize the "neurodegeneration protein" expressed by Fluoro-Jade B positive neurons following neurotoxic insult. The role of apoptosis versus necrosis as a pathway of neuronal death will be more clearly defined with the use of the cytotoxic marker, Fluoro-Jade B and proteomic approaches. The NMDA receptor complex as a mediator of developmental neurotoxicity will be the focus of a new protocol under development in collaboration with CDER. This protocol will specifically determine whether the ketamine-induced neuronal apoptosis observed in the developing rat is also observed in the immature nonhuman primate, an animal model more closely related to the developing human. Control and ketamine-treated animals will be assessed using histochemical, functional, genomic and proteomic approaches whenever possible.

Public Health Significance

Over the last decade, expertise, equipment and facilities have been woven together to pursue the overall goals of neurotoxicology research through three primary research areas. These focal areas were developed and based on prevailing scientific understanding and the importance of each area to regulatory concerns. They include mechanistically based approaches for defining and understanding the potential for a broad range of drugs and other chemicals to produce neurotoxic effects during developmental, adult, or senescent life stages.

Staff will build on our strong base of dose-dependent biomarkers of effect and our unique assessment tools to focus on mechanistically based and fundamental research projects. The use of DNA array expression and proteomic tools will be further developed. Key personnel will be recruited and extensive training will be provided for existing staff so that new technologies can be incorporated into our research approach.

An interdisciplinary approach, the use of multiple established animal models and innovative biomarkers, and an in-depth working knowledge of and experience with mechanistically based focal areas of research enable the Division of Neurotoxicology to be responsive to FDA regulatory needs in a timely fashion. Several ongoing or planned studies, many in conjunction with other FDA centers, exemplify the application of the group's approach to providing critical research information applicable to FDA's regulatory concerns.

Research Projects

Title	Project Number	Strategic Research Goal

PI: Ali, Syed

- **Effects of Ibogaine on Neurotransmitter Systems, Generation of Free Radicals and Nitric Oxide Synthase Activity: Correlation with Neurohistological Evaluations in Mouse and Rat Brain** — **E0698301** — **Agent-Driven**

  Objective(s):

  1) Determine the effects of ibogaine on dopamine, serotonin and their metabolite concentrations in different regions of mouse and rat brain;
  2) Determine the effects of ibogaine on reactive oxygen species (ROS) and lipid peroxidation in different regions of mouse and rat brain;
  3) Determine the effects of ibogaine on the activities of several antioxidant enzymes superoxide dismutase, catalase, glutathione peroxidase and glutathione levels in different regions of mouse and rat brain;
  4) Evaluate the effects of ibogaine on the activity of nitric oxide synthase (NOS) in different regions of mouse and rat brain;
  5) Determine the levels of ibogaine, noribogaine and neurohormone, prolactin and corticosterone in plasma of mouse and rat; and,
  6) Evaluate the neurohistological effects of ibogaine in different brain regions in the mouse and the rat, and correlate them with any neurochemical alterations.

- **ADDEND: The Effects of Ibogaine on Neurotransmitter Systems, Generation of Free Radicals and Nitric Oxide Synthase Activity: Correlation w/Neurohistological Evaluations in Mouse and Rat Brains** — **E0698311** — **Agent-Driven**

  Objective(s):

  Investigate if direct infusion of compounds into the brain produces similar changes in the neurotransmitter system in rats. Inject ibogaine, noribogaine and the structurally related compound harmaline directly into the brain and evaluate the changes in neurotransmitter levels.

- **ADDEND: The Effects of Ibogaine on Neurotransmitter Systems: Correlation with Body Temperature and Electroencephalogram (EEG)** — **E0698321** — **Agent-Driven**

  Objective(s):

  Investigate what effect ibogaine might have on the electroencephalogram profile along with the time course of temperature changes in rats exposed to this compound. Plan to inject ibogaine 50 mg/kg, i.p. in five male adult Sprague-Dawley rats instrumented for the EEG and temperature recording as described in the protocol P00404.

Title	Project Number	Strategic Research Goal

- **Acute Toxicity of Iron Compounds in Young Mice and Rats** **E0703801** **Agent-Driven**

  Objective(s):

  1) Compare acute toxicity in young animals using two forms of iron commonly used in iron supplements and one form that is to be used in fortification;
  2) Determine if high doses of iron compounds produce reactive oxygen species, an alteration in the lipid peroxidation and changes in antioxidant enzymes in different regions of brain and liver of young mice and rats;
  3) Determine the effect of high doses of iron compounds on complete blood counts. CBC, MCV, MCHC, TIBC and the distribution of iron and iron-binding proteins in different regions of brain and other visceral organs in young animals;
  4) Determine if high doses of iron compounds produce significant changes in neurotransmitter concentrations and activity of nitric oxide synthase in different regions of brain in young mice and rats; and,
  5) Determine if high doses of iron compounds produce pathological alteration in brain and other visceral organs in young mice and rats.

Title	Project Number	Strategic Research Goal
◆ **Evaluation of Novel Genetic Changes and Post-Translational Modification in the Protein Products of Specific Genes in Parkinson's Disease and in Substituted Amphetamine Neurotoxicity using Quantitative Proteome Analysis in Mice Models and Human Subjects**	**E0712101**	**Agent-Driven**

Objective(s):

1) Determine the post-translational protein modifications in the protein extracts of nigral and striatal tissues in substituted amphetamines and MPTP-treated mice;
2) Evaluate the effect of various nNOS inhibitors and peroxynitrite decomposition catalysts on the post-translational protein modifications in the protein extracts of nigral and striatal tissues in substituted amphetamines and MPTP-treated mice;
3) Determine the protein-DNA interaction in the nuclear extracts from the nigral and striatal tissues in substituted amphetamines and MPTP-treated mice for the evaluation of novel post-translational changes in the proteins mediated by various transcription factors;
4) Determine the effect of various nNOS inhibitors on substituted amphetamine and MPTP-induced free radical production and monoamine concentration in mouse brains;
5) Determine the nitrated protein on tyrosine hydroxylase by immunoprecipitation of tyrosine hydroxylase and co-localization of 3-nitrotyrosine in the presence or absence of nNOS inhibitors in order to correlate the physiological effects paradigm with the protein changes paradigm from objectives 1, 2 and 3; and,
6) Determine the post-translational protein modifications in the protein extracts and protein-DNA interaction in the nuclear extracts of nigral and striatal tissues obtained from human subjects of Parkinson's Disease.

Title	Project Number	Strategic Research Goal

PI: Binienda, Zbigniew

- **Metabolic Correlates of the Neurotoxicity Associated with Exposure to the Mitochondrial Inhibitor 3-nitropropionic Acid (3-NPA) in the Rat: The Role of Free Fatty Acids (FFA)** — E0701001 — Concept-Driven

  Objective(s):

  1) Evaluate the acute effects of the mitochondrial inhibitor 3-NPA on brain metabolic activity using elecrophysiological, neurochemical, and neurohistological endpoints:
     a) spontaneous electrical brain activity and averaged visual evoked potentials;
     b) FFA concentration in different brain regions;
     c) brain regional monoamine neurotransmitter concentrations: dopamine, serotonin, and their metabolites;
     d) microscopically detectable neuronal damage; and,
  2) assess the possible neuroprotective effect of L-carnitine in the rat model of 3-NPA-induced histotoxic hypoxia.

- **The Role of Mitochondrial Energy Disruption in the Mechanism of Neurotoxicity: Neurophysiological, Neurochemical, and cDNA Microarray Approaches** — E0711001 — Concept-Driven

  Objective(s):

  1) Define neurophysiological and neurochemical phenotypes associated with brain exposure to 3-NPA and L-carnitine;
  2) Define changes in patterns of gene expression induced by 3-NPA and L-carnitine in the rat brain;
  3) Assess the attenuation of energy deficit by L-carnitine using enzymatic and neurochemical biomarkers of neurotoxicity in the rat model of 3-NPA-induced histotoxic hypoxia; and,
  4) Establish the relationship between 3-NPA-induced physiological, neurochemical phenotypes, and transcriptome profiling in the rat brain model.

Title	Project Number	Strategic Research Goal

PI: Bowyer, John

- **Evaluation of the Neurotoxic Effects and Determination of the Mechanisms of Induction of Limbic Seizures Produced by Amphetamine and Related Compounds** — E0702401 — Concept-Driven

  Objective(s):

  1) Measure the effects of dose and age on the susceptibility of amphetamine-induced limbic-type seizures in three different strains of rat and mouse, and identify areas in the brain, in particular the limbic system, where cell death and neuroplastic changes occur after amphetamine-induced seizures;
  2) Determine the seizure-genic capabilities of amphetamine, phentermine, methylphenidate and ephedrine in rat and mouse, the extracellular brain levels of these compounds necessary to induce seizures, and whether hyperthermia plays a role in the seizure induction;
  3) Determine via brain microdialysis if extracellular glutamate levels are elevated in the limbic system (hippocampal rudiments and piriform cortex) prior to and during seizures induced by amphetamines; and,
  4) Elucidate the role the noradrenergic, as well as the glutamatergic, system plays in seizures generated by amphetamines. Furthermore, begin to determine how agonists and antagonists of these two neurotransmitter systems can potentiate the seizure genesis of amphetamine.

- **Multiple cDNA Array Analysis of the Temporal Changes in mRNA Species after Neurotoxic Events** — E0707301 — Predictive Toxicology

  Objective(s):

  1) Develop the use of cDNA arrays as a means of detecting mRNA changes that are potential indicators of subtle and severe neurodegeneration at time points of several days up to months after neurotoxic insult;
  2) Use cDNA arrays to examine changes in mRNA species that may play a role in changes in the phenotypic expression of neuronal populations in selected brain regions;
  3) Expose both neuronal cell line cultures and the brain *in vivo* to neurotoxic insults, and compare the changes in mRNA in the cultured cells versus specific regions of brain using cDNA arrays; and,
  4) Compare differences in mRNA changes in specific brain regions of adult versus neonatal rats.

Title	Project Number	Strategic Research Goal

PI: Chelonis, John

- **Complex Brain Function in Children as Measured by Performance in the NCTR Operant Test Battery** — **E0703301** — **Agent-Driven**

  Objective(s):

  Administer a battery of automated tests (games) to measure aspects of learning, short-term memory and attention, motivation, time perception, and color and position discrimination.

PI: Ferguson, Sherry

- **ADDEND: A Pilot Study to Assess the Effect of Developmental Genistein Exposure on Sexually Dimorphic Behaviors** — **E0212213** — **Agent-Driven**

  Objective(s):

  Determine whether pre-/neonatal exposure to genistein, a compound with estrogenic properties, will alter imprinting of sex differences in behavior.

- **ADDEND: A Pilot Study to Assess the Effect of Developmental Nonylphenol Exposure on Sexually Dimorphic Behaviors** — **E0212513** — **Agent-Driven**

  Objective(s):

  Determine whether pre-/neonatal exposure to nonylphenol, a compound with estrogenic properties, will alter sex differences in behavior.

- **ADDEND: A Pilot Study to Assess the Effect of Developmental Vinclozolin Exposure on Sexually Dimorphic Behavior** — **E0212613** — **Agent-Driven**

  Objective(s):

  Determine whether pre-/neonatal exposure to vinclozolin, a compound with potential estrogenic properties, will alter sex differences in behavior.

- **ADDEND: A Pilot Study to Assess the Effect of Developmental Ethinyl Estradiol Exposure on Sexually Dimorphic Behaviors** — **E0212913** — **Agent-Driven**

  Objective(s):

  Determine whether pre-/neonatal exposure to ethinyl estradiol, a compound with potential estrogenic properties, will alter sex differences in behavior.

Title	Project Number	Strategic Research Goal
◆ **ADDEND: The Effects of Developmental/Chronic Genistein Exposure over Multiple Generations on Maternal, Play, Mating/Reproductive Behaviors and Neurochemical Measures** **Objective(s):** Determine whether chronic exposure of rats over multiple generations to genistein, a compound with potential estrogenic properties, will alter maternal behavior, play behavior of either sex, the female lordosis response, male mating behavior or the amphetamine-induced release of striatal dopamine, which is known to be estrogen-modulated.	**E0213213**	**Agent-Driven**
◆ **ADDEND: The Effects of Developmental/Chronic Nonylphenol Exposure over Multiple Generations on Sexually Dimorphic Behaviors, and Neurochemical Measures** **Objective(s):** Determine whether chronic exposure of rats over multiple generations to nonylphenol, a compound with potential estrogenic and/or androgenic properties, will alter maternal behavior, the female lordosis response, male mating behavior, sodium solution intake, amphetamine-induced release of the striatal dopamine, or serum levels of testosterone and estradiol in males.	**E0213513**	**Agent-Driven**
◆ **ADDEND: The Effects of Nonylphenol Exposure over Multiple Generations on Cognitive Functions and Hippocampal Structure in Female Rats** **Objective(s):** Determine whether chronic exposure over multiple generations to nonylphenol, a compound with estrogenic properties, will alter performance on learning/memory tasks and/or hippocampal structure in young adult and middle aged female rats.	**E0213521**	**Agent-Driven**

Title	Project Number	Strategic Research Goal

- **Validity of Developmental Cerebellar Stunting in the Rat as a Model for Attention Deficit Hyperactivity Disorder: Behavior and Neurochemistry** — **E0704001** — **Concept-Driven**

  Objective(s):

  1) Identify treatments which cause developmental cerebellar stunting, specifically those which decrease the granule cell population with few effects on Purkinje cells;
  2) Confirm the increase in locomotor activity caused by developmental cerebellar stunting and determine the degree to which this hyperactivity resembles human ADHD;
  3) Identify other behavioral alterations associated with developmental cerebellar stunting and determine the degree to which these resemble those associated with human ADHD;
  4) Identify the neurochemical alterations in different brain regions resulting from the developmental insult; and,
  5) Compare these neurobehavioral and neurochemical alterations to those exhibited by the most common rodent model of ADHD: the Spontaneously Hypertensive Rat (SHR).

PI: Patterson, Tucker

- **Neurotoxicological and Behavioral Assessment of the Human Immunodeficiency Virus (HIV) Suppressors 2',3'-dideoxycytidine (ddC) and Thalidomide in Rhesus Monkeys** — **E0250201** — **Predictive Toxicology**

  Objective(s):

  Assess the neurotoxicity and neurobehavioral effects of chronic treatment with the anti-HIV agents 2',3'-dideoxycytidine (ddC) and thalidomide in rhesus monkeys.

Title	Project Number	Strategic Research Goal

PI: Paule, Merle

- **Development of a Nonhuman Primate Model for Studying the Consequences of Long-term Anticonvulsant Medication on Complex Brain Functions (97032)- ASTRA CRADA** — **E0280001** — **Predictive Toxicology**

  Objective(s):

  1) Establish acquisition curves for several operant behaviors in juvenile rhesus monkeys during chronic oral exposure to two anticonvulsant agents and vehicle;
  2) Determine whether such exposure results in any significant changes in the acquisition and performance of these operant and other observable behaviors;
  3) Determine whether such exposure results in any significant changes in clinical chemistry or ophthalmic parameters; and,
  4) Determine plasma distribution profiles and concentrations for each of these agents at various stages of chronic exposure.

- **ADDEND: Development of a Nonhuman Primate Model for Studying the Consequences of Long-term Anticonvulsant Medication on Complex Brain Functions** — **E0280011** — **Predictive Toxicology**

  Objective(s):

  Determine whether the effects of chronic ramacemide treatment are due to reversible effects linked to daily drug exposure or are due to irreversible CNS toxicity. Monitor behavioral acquisition in subjects during six months of reduced drug exposure.

- **ADDEND: Development of a Nonhuman Primate Model for Studying the Consequences of Long-Term Anticonvulsant Medication on Complex Brain Functions – Rodent Equivalent** — **E0280041** — **Predictive Toxicology**

  Objective(s):

  1) Examine the effects of acute and chronic exposure to dizocilpine and/or phenytoin on neuronal degeneration and cell death;
  2) Establish acquisition curves for several operant behaviors in rats during chronic oral exposure to two different anticonvulsant agents;
  3) Determine whether such exposure results in any significant changes in the acquisition and performance of these operant behaviors; and,
  4) Address the relationship between drug-induced cell death and drug-induced changes in behavioral acquisition.

Title	Project Number	Strategic Research Goal

- **ADDEND: Development of Nonhuman Primate Model for Studying the Consequences of Long-term Anticonvulsant Medication on Complex Brain Functions (97032) Rodent Equivalent: Estrous Cycle Assessment and Tissue Collection** — **E0280051** — **Agent-Driven**

  Objective(s):

  1) Determine whether disruptions in reproductive function might also have been affected in previous experiment (E0280041). Daily estrous cycle assessments will be made over a three-week period to determine whether the cycles of experimental subjects differ from those of controls; and
  2) Assess phenytoin blood levels from stored samples collected throughout the previous study. Use blank rat plasma to serve as an analytical matrix for HPLC analysis.

- **Effects of Prenatal Cocaine on Behavioral Plasticity** — **E0663307** — **Agent-Driven**

  Objective(s):

  Determine whether chronic exposure to cocaine *in utero* results in long-term or residual functional consequences in rhesus monkey offspring as adults. Systematically explore how long affected subjects must be exposed to specific reinforcement contingencies before reversals of those contingencies manifest as behavioral problems.

- **Effects of Chronic Methylphenidate (Ritalin) Administration on 'cognitive' Functions in the Rhesus Monkey** — **E0683700** — **Agent-Driven**

  Objective(s):

  Determine whether chronic treatment with relevant doses of the therapeutic agent methylphenidate (Ritalin) will result in detectable changes in specific "cognitive" abilities in a nonhuman primate model of complex brain function.

- **Use of the NCTR Nonhuman Primate Operant Test Battery (OTB) as a Predictor of Acute Neurobehavioral Toxicity: Pharmacological Manipulation at Specific Neurotransmitter Receptor Subtypes** — **E0697901** — **Predictive Toxicology**

  Objective(s):

1) Explore further the extent to which the use of operant behavioral techniques in nonhuman primates can serve to reliably model the effects of compounds selected to act on specific neurotransmitter systems;
2) Determine the acute dose-effect relationships of several drugs believed to act primarily at subtypes of specific neurotransmitter receptors using rhesus monkey OTB performance;
3) Characterize the relative sensitivities of the various behavioral endpoints in the NCTR OTB to pharmacological manipulation of specific neurotransmitter systems and to add new tasks to the NCTR OTB;
4) Characterize more thoroughly the role of specific neurotransmitter systems in the expression of complex brain functions through the pharmacological manipulation of specific receptor subtypes of some of the known major neurotransmitter systems; and
5) Determine if the acute behavioral effects of the exogenous compounds of interest differ with regard to gender in the rhesus monkey.

- **Pharmacological Countermeasures for Space Motion Sickness** — **E0712401** — **Predictive Toxicology**

  Objective(s):

  Establish effectiveness and quantify side effects for potential drug countermeasures for Space Motion Sickness (SMS).

- **Develop Additional MBS Capabilities by ADP Staff - T.O. #498, 668 and 698.** — **P00384** — **Center Support**

  Objective(s):

  Develop new titrated IMP task. This task will be similiar to the IMP task currently being developed except for the time delay which will be increased for each long and short delay. Plans are being examined to implement this new task into the IMP which is currently being designed.

- **Arkansas Children's Hospital Statistical Support - T.O. 22, 586 and 705** — **P00386** — **Predictive Toxicology**

  Objective(s):

  Investigate empirically the OTB performance by normal children and children identified as expressing specific clinical diagnoses including Attention Deficit Disorder with or without Hyperactivity.

Title	Project Number	Strategic Research Goal

PI: Popke, Jon

- **Validation of the NCTR Rodent Operant Test Battery as an Adjunct to the NCTR Primate Operant Test Battery: Implications for the Areas of Risk Assessment and Prediction of Neurobehavioral Toxicity** — **E0691401** — **Predictive Toxicology**

  Objective(s):
  1) Determine the acute effects of a variety of prototypic psychotropic agents on rodent performance in an operant test battery (OTB) containing tasks designed to model several complex brain functions;
  2) Determine the relative sensitivities of the behavioral endpoints monitored in the rodent OTB to pharmacological disruption;
  3) Compare and contrast the acute effects of these psychotropic agents on rodent and primate OTB performance to determine the degree to which behavioral findings in rodents can be extrapolated to primates;
  4) Validate the use of rodent operant performance as useful predictors of neurobehavioral toxicity; and,
  5) Add to existing knowledge of the neurochemical and neurophysiolgoical basis of complex brain functions.

PI: Scallet, Andrew

- **ADDEND: Neurotoxicological Effects of Exposure to Estrogenic Compounds during Development: II. Genistein** — **E0212215** — **Agent-Driven**

  Objective(s):
  1) Determine whether developmental exposure to genistein may modify the sexually dimorphic areas of the adult rodent brain; and,
  2) Compare neurochemical and neurohistological biomarkers of genistein exposure for their relative sensitivity and concordance.

- **ADDEND: Neurotoxicological Effects of Exposure to Estrogenic Compounds during Development: III. Nonylphenol** — **E0212515** — **Agent-Driven**

  Objective(s):
  1) Determine whether developmental exposure to nonylphenol may modify the sexually dimorphic areas of the adult rodent brain; and,
  2) Compare neurochemical and neurohistological biomarkers of nonylphenol exposure for their relative sensitivity and concordance.

- **ADDEND: Neurotoxicological Effects of Exposure to an Anti-Androgenic Compound during Development: IV. Vinclozolin** — **E0212615** — **Agent-Driven**

Title	Project Number	Strategic Research Goal

Objective(s):

1) Determine whether developmental exposure to vinclozolin may modify the sexuall dimorphic areas of the adult rodent brain; and,
2) Compare neurochemical and neurohistological biomarkers of vinclozolin exposure for their relative sensitivity and concordance.

- **ADDEND: Neurotoxicological Effects of Exposure to Estrogenic Compounds During Development: V. Ethinyl estradiol** — E0212915 — Agent-Driven

Objective(s):

1) Determine whether developmental exposure to ethnyl estradiol may modify the sexually dimorphic areas of the adult rodent brain; and,
2) Compare neurochemical and neurohistological biomarkers of ethinyl estradiol exposure for their relative sensitivity and concordance.

- **ADDEND: Multigenerational Exposure to Estrogenic Compounds: I. Genistein Effects on Volume of the Sexually Dimorphic Nucleus** — E0213215 — Agent-Driven

Objective(s):

Evaluate the hypothesis that multigenerational exposure to genistein may produce a reduction in the volume of the male sexually dimorphic nucleus of the medial preoptic area of the hypothalamus.

- **ADDEND: Multigenerational Exposure to Estrogenic Compounds: II. Nonylphenol Effects on Volume of the Sexually Dimorphic Nucleus** — E0213515 — Agent-Driven

Objective(s):

Evaluate the hypothesis that multigenerational exposure to nonylphenol may produce a reduction in the volume of the male sexually dimorphic nucleus of the medial preoptic area of the hypothalamus.

- **Estimating Quantitative Neurotoxicity Risk from Domoic Acid Exposure** — E0693001 — Agent-Driven

Objective(s):

Correlate pharmacokinetic profiles of single and multiple doses of domoic acid with associated quantitative neurohistological and behavioral effects in non-human primates:

(a) Identify genetic factors modulating domoic acid sensitivity in Wistar rats; and,
(b) Identify neurochemical biomarkers of domoic acid exposure and damage.

Title	Project Number	Strategic Research Goal

PI: Schmued, Laurence

- **Development and Validation of a Neurohistochemical Test Battery for Resolving the Distribution of Lesions and the Underlying Mechanisms of Action of Neurotoxicants.** — **E0701301** — **Predictive Toxicology**

  Objective(s):

  1) Develop and validate a battery of conventional and novel histochemical techniques for resolving the nature, distribution and underlying mechanisms of brain damage resulting from exposure to FDA-relevant neurotoxicants;
  2) Localize throughout the central nervous system histochemical and pathological changes resulting from exposure to different classes of neurotoxicants; and,
  3) Correlate a compound's putative mode of action with a characteristic histochemical profile to develop the ability to predict the neuroanatomical regions at risk and the potential functional consequences of exposure to the neurotoxicant of interest.

- **ADDEND: Development and Validation of a Neurohistochemical Test Battery for Resolving the Distribution of Lesions and the Underlying Mechanisms of Action of Neurotoxicants.** — **E0701311** — **Predictive Toxicology**

  Objective(s):

  Addendum submitted to add compound strychine to be used in this project.

- **ADDEND: Development and Validation of a Neurohistochemical Test Battery for Resolving the Distribution of Lesions and the Underlying Mechanisms of Action of Neurotoxicants.** — **E0701321** — **Predictive Toxicology**

  Objective(s):

  Add formaldehyde-fixed human brain autopsy tissue to the list of neurotoxicant exposed brains in the test battery associated with this project.

- **ADDEND: Development and Validation of a Neurohistochemical Test Battery for Resolving the Distribution of Lesions and the Underlying Mechanism of Action of Neurotoxicants** — **E0701331** — **Predictive Toxicology**

  Objective(s):

  Add compounds to the histochemical test battery: Aurothioglucose, Pilocarpine, and Beta-amyloid peptide fragment 1-40.

PI: Slikker, William

- **Quantitative Procedures for Neurotoxicity Risk Assessment** — **E0310001** — **Predictive Toxicology**

Title	Project Number	Strategic Research Goal

Objective(s):

Determine the necessary parameters for a biologically based dose-response model to predict neurotoxic adverse effects following exposure to cholinesterase inhibiting pesticides. Such information would improve the ability of risk assessments to evaluate toxicological data for potential human health risk and address a specific need identified by the Neurotoxicity Risk Assessment Guidelines.

- **Preliminary Studies for the Effects of Chronic Dexfenfluramine Administration in the Rhesus Monkey** — E0702601 — Predictive Toxicology

Objective(s):

1) Determine if the rhesus monkey demonstrates cardiac valve changes due to chronically administered dexfenfluramine; and,
2) Determine if the rhesus monkey demonstrates neurobiological changes due to chronically administered dexfenfluramine.

PI: Xu, Zengjun

- **Adolescent Nicotine Administration Effects on CNS Serotonergic Systems** — E0709801 — Agent-Driven

Objective(s):

1) Determine whether adolescent nicotine administration elicits axonal/terminal damage in 5HT systems;
2) Determine if adolescent nicotine administration alters 5HT presynaptic activity;
3) Determine 5HT receptor and signaling activity and functions induced by adolescent nicotine exposure; and,
4) Determine if adolescent nicotine administration produces changes in cAMP-medicated signal transduction, 5HT metabolic enzymes and/or 5HT receptors.

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Concept Papers

Title	Project Number	Strategic Research Goal

PI: Ali, Syed

- **Neurotoxicity Assessment of Substituted Amphet- amines** — **E0708801** — **Agent-Driven**

Objective(s):

1) Evaluate the neurotoxicity of the eight most popular ephedra-containing dietary supplements sold in the market place and consumed by the public;
2) Determine the IC-50 of these dietary supplements using PC-12 cultured cells;
3) Determine if *in vitro* exposure to these dietary supplements selectively induces specific genomic changes in PC-12 cultured cells using cDNA arrays;
4) Determine if multiple doses of these dietary supplements selectively induces specific genomic changes in different regions of mouse brain using cDNA arrays;
5) Determine if multiple doses of these compounds produce significant changes in neurotransmitter concentrations in different regions of brain in mice;
6) Determine if multiple doses of these compounds produce significant changes in the formation of 3-nitrotyrosine, an *in vivo* biomarker for oxidative stress, in different regions of mouse brain;
7) Determine if multiple doses of these dietary supplements produce reactive oxygen species, alteration in the lipid peroxidation, and changes in antioxidant enzymes in different regions of the mouse brain; and,
8) Determine if multiple doses of these dietary supplements produce pathological alteration in brain and other visceral organs in mouse.

Title	Project Number	Strategic Research Goal

PI: Ferguson, Sherry

- **Assessment of Depression Risk Associated with Accutane (13-cis-retinoic acid or isotretinoin) and al-trans-retinoic acid treatment: Measurement of Behavioral and Neurochemical Alterations in Adult Sprague-Dawley and Flinders Sensitive and Insensitive Line Rats** — E0714501 — Agent-Driven

Objective(s):

1) Describe the behavioral alterations associated with chronic 13-cis-retinoic acid and all-trans-retinoic acid treatment in adult male and female Sprague-Dawley rats;
2) Determine if such alterations resemble those described in humans treated with 13-cis-retinoic;
3) Measure sex differences in behavioral response to 13-cis-retinoic acid and all-trans-retinoic acid treatment;
4) Evaluate the reversibility of the 13-cis-retinoic acid induced and/or all-trans-retinoic acid-induced alterations;
5) Assess if genetic predisposition to depression determines the frequency and/or magnitude of the behavioral alterations associated with 13-cis-retinoic acid and/or all-trans-retinoic acid treatment; and,
6) Quantitate the neurochemical alterations induced by 13-cis-retinoic acid and/or all-trans-retinoic acid treatment.

PI: Jakab, Robert

- **Concept - Genetic Profiling of Regenerating and Degenerating Neurons after Amphetamine-Neurotoxicity** — E0713401 — Predictive Toxicology

Objective(s):

1) Determine the gene expression profile of three cell groups uniquely susceptible to amphetamine exposure;
2) Determine the first appearance of the three affected cell groups and the pattern and timetable of their density changes;
3) Compare the genetic profiles of adjacent cortical neurons susceptible and resistant to amphetamine;
4) Compare the genetic profiles of *de novo* striatal TH* neurons, adjacent TH* neurons, and midbrain TH* neurons; and,
5) Search for genes, mRNA species, and proteins - candidates for potential pharmacological manipulations that can aid the recruitment of striatal neurons to produce and release DA.

Title	Project Number	Strategic Research Goal

PI: Patterson, Tucker

- **Analyses of the Rat Hippocampus via DNA Microarrays and a Novel Antibody Array, coupled with Laser Capture Microdissection (LCM) - Evaluation of the Effect of Aging on Gene and Protein Expression Associated with Learning** — E0713901 — Concept-Driven

  Objective(s):

  1) Measure gene and protein expression in regions of the hippocampus to determine regional distribution;
  2) Determine the effect of aging on regional distribution of hippocampal proteins in three strains of rats;
  3) Determine if aging, behavioral performance, and alterations in gene and protein expression in the hippocampus are related; and,
  4) Correlate the differences in gene and protein expression with behavioral performance of young adult and aged rats in learning tasks previously shown to be sensitive to changes in protein expression.

PI: Paule, Merle

- **Concept - Automated Cognitive Assessment of Persons with Alzheimer's Disease** — E0715301 — Predictive Toxicology

  Objective(s):

  Assess the OTB performance of 20 AD patients and 20 age-matched control subjects to determine: the applicability of such procedures in elderly persons; whether OTB performance is different between persons with AD and age-matched controls; which OTB task(s) is (are) most sensitive to disease severity; and to compare OTB performance with that from other clinical instruments for cognitive assessment used routinely at the Alzheimer's Disease Center.

PI: Scallet, Andrew

- **Neurotoxicology of Hormone Replacement Therapeutics** — C20005 — Predictive Toxicology

  Objective(s):

  Investigate the effects of genistein and Premarin on hypothalamic neuropeptides using classical methodologies as well as newer genomic and proteomic approaches.

Title	Project Number	Strategic Research Goal

PI: Schmued, Laurence

- **Concept - Proteomics of Toxicant Induced Neuronal Degeneration** — **E0711101** — **Predictive Toxicology**

Objective(s):

1) Resolve the chemical identity of the endogenous protein(s) associated with neuronal cell death as identified by Fluoro-Jade B binding;
2) Determine if the same proteins are expressed regardless of the mechanism of neurodegeneration;
3) Resolve the metabolic pathway by which the "degeneration protein" is generated; and;
4) Resolve the chemical identity of the fluorescent component in Fluoro-Jade B responsible for the high affinity labeling of degenerating neurons.

Veterinary Services

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Division of Veterinary Services provides research support through on-site contractor for histopathology.

Executive Summary

Introduction

The Division of Veterinary Services (DVS) provides professional and technical support to the various NCTR research divisions and Centers of Excellence in their efforts to conduct peer-reviewed scientific research that supports and anticipates the FDA's current and future regulatory needs. The Division provides administration for the Center's Animal Care and Use Program, which is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC International). Included within the Division are the contracted services for animal care, diet preparation, and pathology, all of which are staffed by on-site contract employees.

FY 2002 Accomplishments

Immediate Office

During 2002, the Division provided oversight and veterinary management of all laboratory animals and housing facilities at NCTR. Divisional personnel consulted with investigators in the establishment of two transgenic mouse breeding colonies in support of a study on photoinduction of cutaneous malignant melanoma in a mouse model. Breeding animals from our colony of obese mice were sent to several other institutions in efforts to establish breeding colonies of those animals at those institutions. Divisional personnel were responsible for breeding, rearing, and/or acquiring all experimental animals used on-site. Divisional personnel completed and submitted annual reports assuring compliance with Federal regulations and NIH guidelines relative to the Animal Care and Use Program. Personnel participated in semi-annual program reviews, facility inspections, and experimental protocol reviews as part of the NCTR Institutional Animal Care and Use Committee proceedings. The director was part of a small group that consulted with architects, engineers, veterinarians, and facility managers of select BSL-3 facilities to facilitate the planning of a BSL-3 animal facility at NCTR. The director serves as a member of the FDA Research Animal Committee and its AAALAC International accreditation subcommittee which performs peer reviews of each center's Program Description Document and performs "mock" AAALAC site visits of the centers prior to actual site visits by AAALAC representatives. The director is also a member of the interagency working group on nonhuman primate resources. Divisional personnel serve as government project officers for the pathology

services, animal care and diet preparation, and rodent bedding contracts. During 2002, divisional personnel planned, organized, and accomplished the annual activities associated with the National Laboratory Animal Care Technician Recognition Week. Divisional personnel participated as instructors in the on-site technician certification program authorized by the American Association for Laboratory Animal Science (AALAS). Divisional personnel also planned, organized, and accomplished activities associated with the 2002 Annual Meeting of the Arkansas Branch AALAS that was held at NCTR.

Animal Care/Diet Preparation Services

During 2002, the average number of experiments supported per month by contract animal care personnel was 25. These experiments entailed as a minimum the daily animal care support of an average of 5,000 rodents and 85 rhesus monkeys. Technical manipulations for these studies included one or more of the following procedures: tattooing (8,400 animals), vaginal lavages (12,000), tumor palpations (20,000), injections (7,800 SQ, IM or IV), oral gavage (7,200), behavioral testing (30,000), and blood collection (2,500). Contract diet preparation personnel provided consultation and nutritional support and diet preparation for several carcinogenicity studies including malachite green, leucomalachite green, and Aloe vera, and endocrine disruptor studies including ethinyl estradiol, genistein, and daidzein, funded through the Interagency Agreement with the National Institute for Environmental Health Sciences (NIEHS). Personnel submitted two manuscripts for publication in the scientific literature. During 2002, diet preparation personnel produced dosed diet, autoclaved routine rodent diet, produced dosed water, and sized dietary pellets for use in caloric restriction studies. Quality assurance personnel performed 4,800 quality control audits of contractor-performed procedures and updated all animal care and diet preparation SOPs.

Pathology and Pathology-related Services

During 2002, four trainees completed PAI's Laboratory Technician apprenticeship training program and became eligible to take the histotechnician registry exam. Ten new apprentices were recruited and hired to start a new training cycle that began in October 2002. Personnel began using Microsoft Project to monitor progress of specimens through pathology. Data slides were prepared for pathology personnel and other NCTR researchers using PowerPoint and a Polaroid ProPalette 7000 Film Recorder with developing being accomplished with a Jobo Autolab Automatic 35mm processor. Pathology personnel continued to work with the information technology (IT) contractor to finalize the "paperless" pathology system for collecting and reporting of pathology data and tracking of specimens through the pathology system. Personnel cleaned out the supply storage in Building 5B and discarded unusable materials. Outdated laboratory supplies/equipment were either discarded or distributed to local high schools for their use. The electron microscope laboratory was dismantled with all equipment either salvaged or donated to universities and high schools. Personnel worked with local IT personnel and those from NIEHS/NTP to transfer pathology data for studies on urethane, fumonisin, chloral hydrate and malachite/leucomalachite green from NCTR to the NTP. Pathologists converted to NTP's LDAS system for data collection for NTP studies.

New equipment for the histopathology laboratory included a tissue processor, a stainer, embedding stations, microtomes, and microscopes. Laser capture microdissection equipment was procured and a laboratory established to support those types of procedures at NCTR. A tissue micro-array system was procured to support ongoing and proposed research in this newly developing field. The old photomicrography system was replaced in 2002 with a state-of-the-art fluorescent (bright field) photography system.

Personnel provided technical support for completion of glutamate synthase assays and *in situ* hybridization work for several research divisions. Technicians were trained in methods of preparation and interpretation of vaginal cytology specimens in support of the endocrine disruptor studies at NCTR. Administrative personnel have prepared for the QA and Pathology Working Group reviews of the malachite green and leucomalachite green NTP-supported studies that are to be held at NCTR in the Fall of 2002.

During 2002, pathology contract employees authored or co-authored 27 publications or presentations.

FY 2003 Plans

- Continue to support the research mission of NCTR, seeking ways to become more efficient in our efforts.
- Establish a BSL-3 animal facility at NCTR and equip the NCTR animal quarantine with equipment that will allow more efficient space utilization.
- Continue supplying methods development and support, both technical and professional, needed to accomplish the NIEHS IAG work at NCTR.
- Continue a quality laboratory animal care program that is consistent with State and Federal laws, regulations, and guidelines.
- Continue to assist FDA's centers in maintaining accreditation of their laboratory animal care and use programs by AAALAC.

Public Health Significance

FDA's mission is to protect and promote the nation's public health. Animal-related studies such as those being conducted by the NCTR research community greatly enhance the Agency's ability to meet this public health mission. The Division of Veterinary Services (DVS) has the facilities, equipment and personnel to actively support this vital interdisciplinary research.

The "gold standard" for laboratory animal care and use programs is accreditation by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International). Such accreditation is widely accepted by the scientific community and indicates that the accredited organization conforms with all government policies and regulations and that it endorses the highest quality care for the animals involved in their animal use activities. DVS personnel oversee the NCTR Laboratory Animal Care and Use Program, which has been accredited by AAALAC International since 1977. The DVS director, working through the FDA Research Animal Council (FRAC), has assisted, and will continue to assist

other FDA centers in obtaining and maintaining accreditation of their animal care and use programs.

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Sams, R.L., Couch, L.H., Miller, B.J., Okerberg, C.V., Warbritton, A.R., Wamer, W., Beer, J. and Howard, P., The effects of alpha- and Beta-Hydroxy acids on the edemal response induced in female SKH-1 mice by simulated solar light., *Toxicology and Applied Pharmacology*, Accepted: 7/12/2002 (E0213101)

Project Number Codes:
E–Ongoing P–Preliminary S–Support

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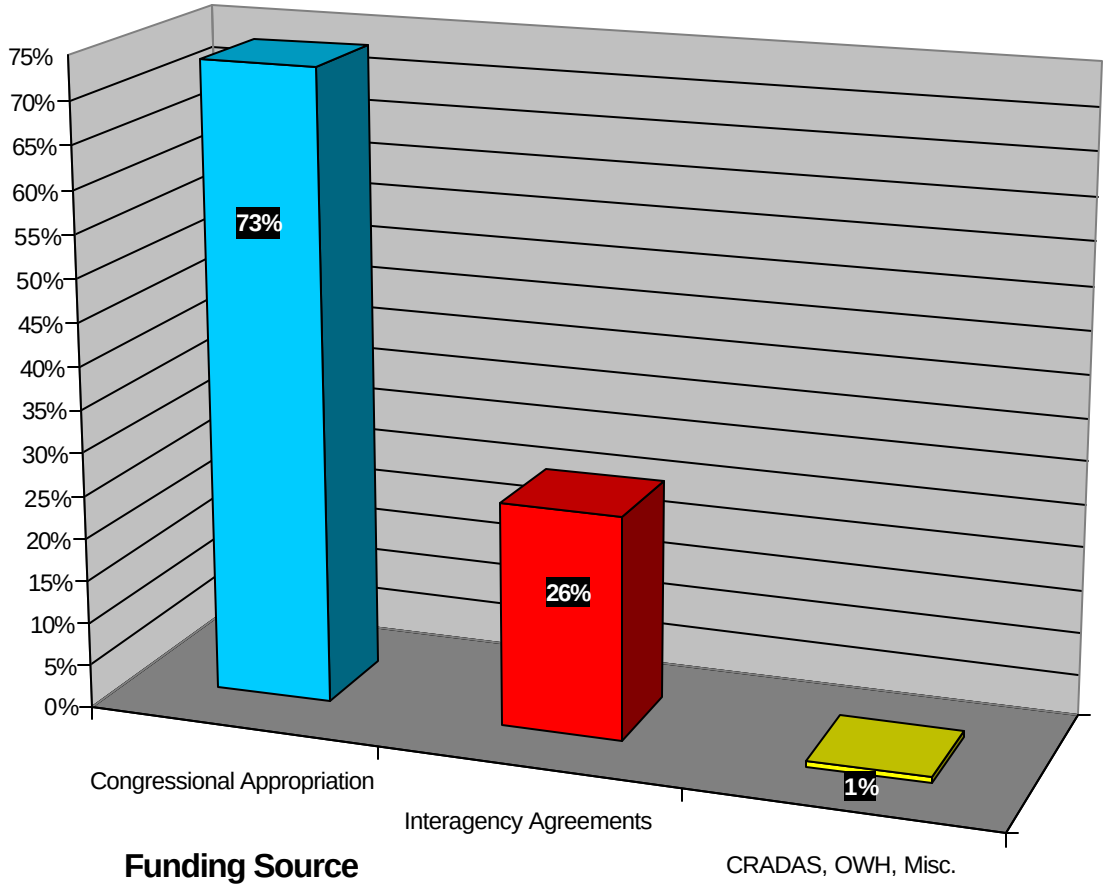
SUMMARY OF
EXTERNALLY FUNDED PROJECTS*

RELATIVE PROPORTIONS OF NCTR BUDGET



*Details of projects presented under individual research division reports.

Interagency Agreements (IAGs)

NCTR has been fortunate in establishing Interagency Agreements (IAGs) with other government agencies to conduct research on problems of common interest to the FDA and the collaborating agency. The most significant, in terms of size, is the IAG between FDA/NCTR and the National Institute of Environmental Health Sciences (NIEHS).

With financial support from the National Toxicology Program (NTP), which is conducted under the auspices of the NIEHS, the NCTR has agreed to conduct animal bioassays, mechanistic studies, and risk assessments on a number of compounds of regulatory interest to both the NIEHS and the FDA. This IAG has allowed NCTR to conduct studies including: a mycotoxin, fumonisin B_1, a study nominated by the FDA Center for Food Safety and Applied Nutrition (CFSAN); the pediatric sedative, chloral hydrate, nominated by FDA's Center for Drug Evaluation and Research (CDER); malachite green, a therapeutic agent used in aquaculture, nominated by FDA's Center for Veterinary Medicine (CVM); and the interaction of ethanol and urethane, nominated by CFSAN. Also, a mechanistic study on riddelliine, a compound of interest to CFSAN, is being supported by the FDA NIEHS IAG. Studies are also beginning on the risk associated with *Aloe vera* exposure in dietary supplements.

Additional research funded via the FDA NIEHS IAG includes a series of studies on several endocrine-active compounds including genistein, ethinyl estradiol, and nonylphenol. The studies will determine the endocrine-disrupting effects of these compounds on reproduction, behavior, and carcinogenesis over multiple generations.

As a result of CFSAN's concern about the potential interaction of ultraviolet (UV) light and over-the-counter cosmetics containing alpha- or beta-hydroxy acids, support for development of a unique Phototoxicology Research and Testing Laboratory at the NCTR was received from NIEHS/NTP. Risk assessments on a number of FDA-regulated products suspected of interaction with sunlight or fluorescent tube-generated light began in FY 1999.

The Environmental Protection Agency (EPA) has supported NCTR in conducting a broad area of research on neurotoxicity risk assessment, risk assessment associated with waterborne and foodborne pathogens, and support for the development of an endocrine disruptor computerized knowledge base.

As an offshoot of a patent and licensing agreement with Cox Recorders dealing with the detection of decomposed food, the Federal Aviation Administration (FAA) has entered into an agreement with scientists at the Center to explore methods of detecting explosives in airline baggage.

The National Institutes of Health (NIH) and the National Cancer Institute (NCI) are supporting studies at the NCTR into Agent Orange exposure and the mechanism of colorectal cancer, respectively.

Although not an IAG in the strict sense, NCTR has received generous support from the FDA's Office of Women's Health (OWH) for a number of research programs. These include: 1) the development of methodologies to assay hydroxylation of endogenous estrogens as that process relates to the risk of developing breast cancer; 2) research on the effects of dietary supplements on women's health issues; and 3) research to develop a human hepatocyte cell line to analyze gender differences in the metabolism of drugs.

NCTR has received support from both the FDA's Office of Women's Health and the U.S. Department of Defense (DOD) to conduct molecular epidemiology studies designed to determine the variability in metabolic phenotype and genotype in women with respect to their recurrence of breast cancer following high-dose radiation and chemotherapy.

Collaborative Research and Development Agreements (CRADAs)

Both the American Chemistry Council (ACC), formerly the Chemical Manu-facturers Association (CMA), and the Environmental Protection Agency (EPA) have provided NCTR support for the development of a computerized predictive Estrogen Knowledge Base (EKB). Using Quantitative Structure-Activity Relationships (QSAR), the EKB will be able to screen chemical structures for estrogen activity. The EKB will also serve as a prototype for predicting activity of chemical classes such as androgens and thyroid hormones, and may be applied to other toxic endpoints such as neurotoxicity and carcinogenesis.

NCTR's Division of Neurotoxicology has received financial support from AstraZeneca to study the effects of long-term blockage of glutamate receptors and/or sodium channel blockage on neurobehavioral endpoints in the non-human primate.

A CRADA with the Arizona Cancer Research Center, University of Arizona, is supporting research into the relationship of dietary habits and colon cancer. Specifically, the objectives of this research are to explore the relationship between dietary isothiocyanates, glutathione S-transferase induction, and colon polyp recurrence.

It has been determined that animals exposed to cocaine during gestation fail to alter their behavior in response to important changes in their environment (i.e., to adapt). Researchers at the University of Arkansas at Little Rock and in the Division of Neurotoxicology at NCTR are expanding upon these findings by examining additional aspects of behavioral plasticity/adaptability by changing 'the rules of the game' for a variety of behavioral tasks.

University Interactions

Many NCTR scientists hold adjunct faculty positions and collaborate with individuals and departments of universities. This practice has been instrumental in leveraging both the intellectual and infrastructure capabilities of NCTR. NCTR scientists have developed research collaborations with more than 20 universities and many scientists have been granted adjunct academic positions. This arrangement permits NCTR staff to develop close collaborative efforts with various university staffs to solve problems of mutual interest to FDA and the respective university. Academic collaborations include mutual use of specialized equipment, sharing of research samples to maximize the gain of information from a project, and the exchange of staff between the institutions for lectures, seminars, and conduct of research.

Of particular importance are the close collaborations between NCTR and the University of Arkansas for Medical Sciences (UAMS) in Little Rock, AR. In addition to the adjunct positions held by NCTR scientists at the UAMS, NCTR participates in the UAMS Interdisciplinary Toxicology Program through which graduate students receive a Ph.D. in toxicology. Many of the graduate students perform research for their dissertations in an NCTR laboratory under NCTR staff supervision.

NCTR is continuing its aggressive leveraging activities by working with the University of Arkansas for Medical Sciences on establishing mutually supportive core DNA/RNA microarray facilities in central Arkansas. Working together, the Microarray Center Directors are establishing consensus milestones that include a timetable for implementation, an agreement on core expertise that will have to be established and how facilities will best be used. The Centers will focus on microarray printing, RNA purification, cDNA labeling, hybridization, scanning, bioinformatics and analysis.

Another example of leveraging with local institutions is that NCTR staff in the Division of Neurotoxicology have access to a behavioral testing laboratory at the Arkansas Children's Hospital (ACH) and at the University of Arkansas at Little Rock (UALR), where results of behavioral studies obtained in animals at NCTR are verified in humans at ACH. In addition, staff members serve as collaborators on a number of grants with area universities. These include a NASA-funded UAMS (Department of Otolarynology) project designed to provide information on the efficacy of several drugs used as anti-space motion sickness therapies and their effects on cognitive function as assessed using the NCTR Operant Test Battery. More recently, the Alzheimer's Research Center in the Center on Aging at UAMS has provided support for studies examining the ability of the NCTR Operant Test Battery to detect and monitor cognitive dysfunction in Alzheimer's patients. A recent CRADA with the UALR provides support via the National Institute on Drug Abuse to study the effects of cocaine exposure during pregnancy on the cognitive abilities of nonhuman primate offspring.

Collaborations by NCTR scientists with universities in the U.S. and abroad have resulted in, at no cost to FDA, a number of visiting scientists who come to NCTR to pursue research in areas developed by NCTR scientists. Thus far, in FY 2002-2003, NCTR has hosted more than 36 visiting scientists from the U.S. and 15 foreign countries. These visiting scientists not only

contribute valuable scientific expertise to NCTR research programs, but many return to their respective institutions to continue research on problems of interest to FDA and NCTR.